

## ISOLATION OF TRICHOMONAS CLONES BY MEANS OF MONOSPORIC ISOLATION APPARATUS

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**Abstract.** A new simple method is described enabling a rapid isolation of trichomonas clones by using the Meopta 59631 type of apparatus designed for monosporic isolation of fungi. From a diluted culture of trichomonads, spread on a thin 2% agar layer, single cells are isolated by means of a hollow puncture knife attached to the objective of a microscope. Afterwards the agar column with the isolated cell is transferred to a microtube containing Diamond's TYM medium in which multiplication of the clones takes place. The adjustment of the puncture knife in the axis of the objective enables a look-through to the material destined for isolation assuring thus a visual control of the clone purity. By means of this method the authors isolated clones of the following species: *Trichomonas vaginalis*, *T. gallinae*, *Tetratrichomonas gallinarum*, *Pentatrichomonas hominis*, *Tritrichomonas suis*, *Tritrichomonas foetus*, *Tritrichomonas augusta* and *Monocercomonas colubrorum*.

For an exact study of the genetic variability of trichomonads their isolation in clones is indispensable. Still a short time ago only unpractical drop dilution methods (e.g. FLICK 1954, JENSEN, HAMMOND 1964) or the micromanipulation were used (REES 1937). A successful cultivation of trichomonads on solid media facilitated the clone isolation on agar plates or in agar columns (SAMUELS, STODER 1960, IVEY 1961, SAMUELS 1962, CAVIER, GEORGES, SABEL 1964). The agar techniques too can however not be considered as optimal, with regard to their following disadvantages: a) there is no visual control of the clone purity. Though in strong dilutions isolated cells occur with great probability, incidental growth of a colony from more than one cell cannot be excluded; b) during inoculation a short-lasting thermal shock (45 °C) sets in which may exercise an unfavourable influence especially on trichomonads from cold-blooded animals. It can be assumed that under such conditions an undesirable selection of cells may take place. c) The procedure is relatively slow and requires high erudition. (The risk of precocious agar hardening during the dilution process, possibility that the serum gets coagulated if the medium is overheated; possibility of errors in the dilution technique which will become manifest only after several days' incubation, difficulties with condensed water.)

We therefore developed a new method using a device designed for monosporic isolation of fungi and produced by Meopta, Prague (Czechoslovak patent No 84005 NEČÁSEK, PALEČKOVÁ, TESAŘ 1953).

## METHOD

Monosporic isolation apparatus Meopta type 59631

MON objective  $10 \times 0.30$  Meopta type 57166

Microscope

Rubber ball with unidirectional valve

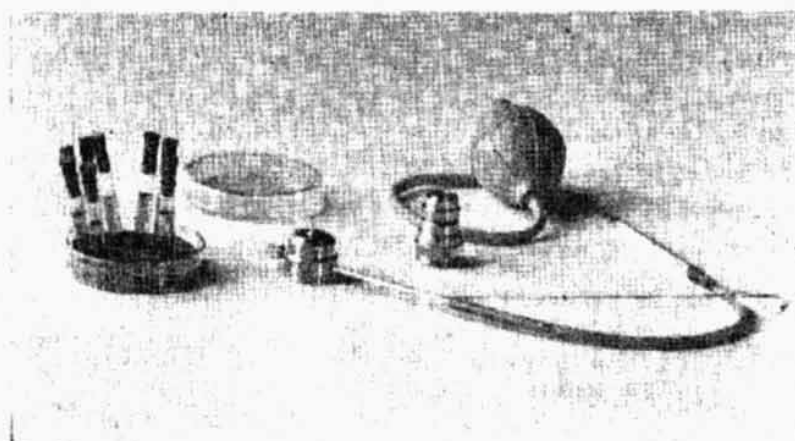
Air-filter with a small connecting rubber tube

Petri dishes (7 cm in diameter) with a sterile 2% agar in a phosphate buffer (pH 7) spread in about a 3 mm layer.

A sterile glass triangle for spreading the material.

Microtubes  $4 \times 0.5$  cm containing sterile TYM medium, closed by rubber stoppers.

The monosporic isolation apparatus has the form of a cylindrical case attached to the MON objective, specially adjusted for this purpose. The bottom of the isolation apparatus extends into a hollow puncture knife of 0.8—0.9 mm inner



**Fig. 1.** Instruments for clone isolation of trichomonads: monosporic isolation apparatus Meopta 59631 connected by means of a small connecting rubber tube with an air-filter and a ball, objective MON  $10 \times 0.30$  Meopta 57166, microtubes containing culture medium, Petri dish with agar and a glass triangle used for spreading the material.

diameter, enabling a look-through to the material destined for isolation. When the object is in focus, the distance between the bottom of the puncture knife and the working level is only 0.5 mm.

From one side of the isolation apparatus protrudes a metal tube to which a ball is connected. Between the ball and the isolation apparatus an air filter (glass tube with cottonwool) is inserted. Before use, the isolation apparatus, the filter and the small connecting tube are sterilized by autoclaving at  $110^{\circ}\text{C}$ . When the isolation apparatus is attached to the objective, a small tightly closed space arises into which the air, necessary to press the agar block out of the puncture knife, is conveyed with the aid of a ball.

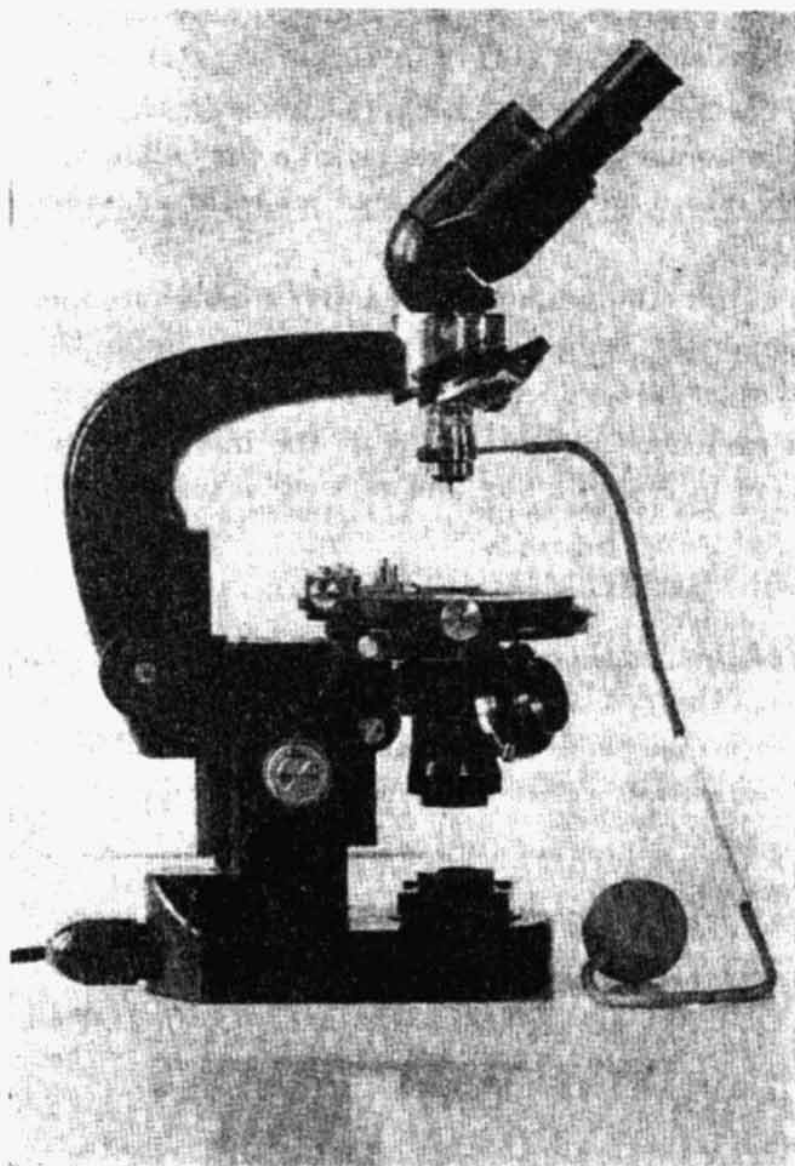


Fig. 2. Monosporic isolation apparatus Meopta 59631 ready for use.

## PROCEDURE

1. A 24-hour-old culture of trichomonads on TYM medium is diluted by physiological saline so as to achieve a final number of organisms—about  $1 \times 10^4$  per ml.
2. 0.5 ml of culture diluted as stated above, is transferred to a mildly dried agar plate and spread in equal layer by means of a sterile glass triangle.
3. The sterile isolation apparatus is attached to the MON objective fitted to the microscope and, with the aid of a small connecting rubber tube, the air-filter and the ball are adjusted to the side-tube of the isolation apparatus.
4. The agar plate with the material is adjusted to the stage of the microscope and a single trichomonad suitable for isolation is found out, making sure that there is no other cell within the field of view.
5. The isolated cell is placed exactly in the middle of the field of view and the body tube of the microscope is put down so that the puncture knife of the isolation

apparatus can cut out an agar column under the isolated protozoon, down to the bottom of the dish.

6. The body tube is slightly lifted and, by means of the ball, the agar block with the isolated trichomonad which remained within the hollow space of the puncture knife, is carefully blown out into a prepared microtube containing TYM medium (Diamond 1957).
7. The microtubes are thoroughly closed by rubber stoppers and incubated at 37 °C (trichomonads from warm-blooded hosts) or at 20 °C (trichomonads from cold-blooded hosts).

To prevent an eventual contamination of the material it is recommended to perform the isolation in a sterile box and to wear a surgical gauze mask.

## VERIFICATION OF THE METHOD

The applicability of this method was tested by isolation of clones of 9 various trichomonas species. The results are summed up in Table 1. Prior to isolation, all the strains used were maintained as axenic cultures on TYM medium (DIAMOND 1957) at pH 7 (*Trichomonas vaginalis* at pH 6) and isolated on the same culture media.

Table 1. Results of experiments with clone isolation in various species of trichomonads

Species and strain	Control terms (in days post inoculation)	Number of isolations	Positive	Negative
<i>Trichomonas vaginalis</i> C 20	5	10	7	3
<i>Trichomonas gallinae</i> H 3	5	10	9	1
<i>Tetratrichomonas gallinarum</i> 4 5	5	10	7	3
<i>Pentatrichomonas hominis</i> TH/A	6	10	7	3
<i>Tritrichomonas foetus</i> KV 1	4	10	9	1
<i>Tritrichomonas suis</i> PC 6	5	10	10	0
<i>Tritrichomonas augusta</i> 102	14	10	9	1
<i>Monocercomonas colubrorum</i> 543	14	10	7	3

The speed of the clone growth in single species as well as in the clones of the same species was different. The most rapid growth was observed in *Tritrichomonas foetus* and *Tritrichomonas suis* clones (opacity visible with the naked eye as early as 2 days after inoculation), slowest growth values were ascertained in *Tritrichomonas augusta* (opacity after 10 to 13 days).

## CONCLUSION

The described technique enables a simple and rapid isolation of trichomonas clones with only a small risk of technical faults. The possibility of a direct visual control guarantees that the isolated lines are actually derived from one single cell. During

the procedure trichomonads are not exposed to unfavourable influences, such as thermal shocks observed in agar techniques. This method, successfully proved in trichomonads, will most probably be applicable also in other groups of Protozoa.

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