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Scope and Method of Study: The report has been prepared for the teachers of the biological sciences. It is the hope of the author to encourage the teacher to collect, culture, and preserve various invertebrate specimens for future classroom demonstration. A science teacher of today should be prepared to take advantage of their natural resources. The purpose of this paper is to introduce ways which will benefit the teachers so they may fulfill their ambitions in the classroom by cultivating the natural surroundings.

It is fully realized that in the scope of the report it is impossible to describe all useful methods for collecting and preserving invertebrates. Considering this fact, only the more common forms are described. The literature for this study came from various biological handbooks and textbooks written by people who have had several years experience with invertebrate zoology.

Findings and Conclusions: The collecting, culturing, and preserving invertebrates will allow the teacher to plan field trips, organize field projects, provide material for the laboratory, and preserve materials for use when living materials are not available. The findings in this report reveal methods of collecting, culturing, and preserving the one-celled Protozoa and related multi-cellular animals of a higher nature. The higher organisms include such phyla as; Porifera, Turbellaria, Aschelminthes, Annelidia, Mollusca, and Arthropoda.

ADVISER'S APPROVAL

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COLLECTING, CULTURING, AND PRESERVING
INVERTEBRATES

By

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INVERTEBRATES

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CHAPTER I

INTRODUCTION

Many times the high school biology teacher is confronted with a problem of not having specimens for demonstration in his classroom. Many demonstrations are conducted in the classroom using material that is not common to the students. This problem might be solved if the teacher would search the surrounding community for live material, living in their natural environment. By doing this he will stimulate interest among his students because he will be presenting material with which they are at least partially familiar. To really appreciate nature one should recognize organisms in their natural habitat; therefore, it would be much better to use specimens from the surrounding ecological community, if possible.

It would be advisable for teacher and students to take many field trips during the warm season. Vast collections could be made and the specimens that haven't been studied could be preserved for use at a later date. A field trip conducted in a useful manner teaches students how to collect and creates enthusiasm.

Perhaps nothing is more frustrating to a biology teacher than to conduct a class without suitable materials. In this report an attempt is made to reveal typical ecological situ-

ations where invertebrates may be found. These are the more common types which could be used in the classroom. Also, this report is designed to aid the teacher in his methods of collecting and preserving such material.

The general organization of this report follows the ladder of classification, from the lower one-celled invertebrates to the higher multi-cellular invertebrates.

Protozoa, being the group of one-celled organisms that are very popular and useful for classroom demonstrations are explained in the first chapter. This chapter is devised to give an explanation of methods for collecting, culturing and preserving the protozoa.

The following chapters are composed of related invertebrates that explain the same thing. These, of course, are the more common types. To explain all methods, and organisms applying to each method, would be far beyond the scope of this report.

CHAPTER II

PROTOZOA

Habitat. Protozoa, with few exceptions, are found in an aquatic environment especially during the active reproductive stage. They may be driven by wind on dust and other particles but are seldom collected from quiet air of rooms. The most abundant sources are in plant remains that have grown in ditches, ponds, or depressions where water is frequently found. Also, protozoa can be found in surface scum, bottom ooze of ponds and scrapings from plants that live in the pond.

Collecting. When material from a pond is collected, bottles with stoppers or jars with lids are needed. If water containing protozoa is present, it is best to fill the container about one-half full and to unstopper the bottles as soon as possible to permit aeration. If dry culture materials are to be collected, sacks, cans, or any kind of container will be suitable to hold the dead grass, leaves, sticks, or other things collected. Add water to the dry material and in a few days you will probably observe that protozoa and other micro-organisms are present.

Culturing. After materials have been collected the preparation of cultures is fairly simple.

Put a quantity of the grass, leaves, etc. into a

container and add enough water to more than cover the material. Allow this to stand in a warm place but not in sunlight. If observed in about 24 or 48 hours the changes in productivity of organism will be greatly excelled. Starting with almost no protozoa to a dense population in from 10 to 15 days and then declining to a very small population. Also, there will be types appearing and disappearing during this time.

Preserving. Sometimes an unusually good or rare culture is discovered and it may be preserved for future use. This material is often used to give the student a better understanding of detail which cannot be seen in the faster moving forms. A living culture is more useful and should not be completely replaced by preserved specimens.

Not all preservatives will preserve all forms but a ten per cent solution of formalin is a very good general preservative. Another good solution which acts as a killing and fixing agent, and will preserve protozoa indefinitely consists of the following:

50 per cent alcohol	90 parts
4 per cent formalin	5 parts
glacial acetic acid	5 parts

The addition of a little glycerin will keep the organisms from becoming too brittle.

When killing and preserving microscopic animals use the following method. Examine a medicine dropper of the culture fluid under a lens to be sure that many protozoa

are present. Put the material into a dish or bottle containing about two to five times as much preservative as you have culture medium. If this is not done the medium may dilute the preservative to the point where it is not effective. It is usually best to run the culture into the preservative. If we put the specimens into a vial or bottle the cork should be dipped in melted paraffin or vaseline to prevent evaporation.

Pure Cultures. In mixed cultures of protozoa there will be certain forms that are abundant for a while and then decline.

Amoebae are not found in mixed cultures of protozoa as might be expected. Occasionally they may be found at the bottom of the container or on the surfaces of leaves and stems. The food for the amoeba consists of minute protozoa and one-celled plants; therefore, food must be available in the medium if a good production is expected. A good medium that could be used for the production of amoebae could be to mix 100 cc. of water (distilled if possible) with three grains of rice. After soaking, contaminate the water with *Saprolegnia* (water mold, a fungus found in nearly all ponds and streams) and then put in some dead grass and leaves that might be abundant with protozoa. Let the container set in a warm place for about a week. The amoebae should be so abundant as to cover the bottom of the container. New cultures should be started about every three or four weeks.

Paramecia are probably the easiest of protozoa to reproduce in a laboratory or class room. First, one should collect some dead grass and other debris, and boil it in water for twenty minutes. Then divide the solution into separate bottles or test tubes and let it set open until bacteria are apparent. A good sign is the formation of a faint scum on the surface. Using a pipette draw a small amount of culture medium from one of the stock bottles that contains a number of paramecia. Picking out one paramecium from a mixed culture may be very difficult but it can be accomplished in the following manner: Put a microslide under a microscope, adjust the scope to low power, then with the micropipette place a few drops of the culture medium on the slide. Look carefully and you will see a paramecium in one or more of the droplets. Clean the micropipette by placing it in hot water and then into cold water. Capillary attraction will allow the droplet containing a paramecium to adhere to the pipette while transferring it to a glass containing a culture medium. Simply blow the organism away from the pipette and into the culture medium. This method should be used for each transfer. After inoculation the tubes of media are stored away for about ten days, but check them at one or two day intervals. Now we have a pure culture that descended from one ancestor. The students will enjoy making these cultures and it will be a good problem for them. It may be possible for them to see dividing individuals but if they can't it is obvious that asexual repro-

duction took place.

Sometimes it is desirable to study a chlorophyll-bearing organism in class. If this is true, *Euglena* makes a very good specimen and can be cultured in the following method. Place about one gram of sheep manure or dry chicken manure into a container and cover it with 250 cc. of water and boil for ten minutes. After cooling let it stand open to the air or blow some dust into it so that it will become contaminated with bacteria. When 36 to 48 hours have passed it is ready for inoculation with euglenae. They will increase rapidly and cause the medium to become green in appearance. The euglenae are sometimes found in pure cultures in ponds and ditches. This is especially true if the water appears greenish in color. Examine carefully because algae can also make water a greenish color. (Miller and Blaydes, 1938)

CHAPTER III

FRESH-WATER SPONGES

Habitat. All sponges are aquatic and mostly marine, but there are a few forms that live in fresh water such as ponds, lakes, and streams. They are not easily seen but will appear as brownish slimy blotches on sticks, stones, and other objects submerged in the water. Their size ranges from a fraction of an inch to more than an inch in diameter. They are very hard to recognize unless one is looking especially for them and knows what to look for.

Collecting. Collecting can be done with a can or jar but must be put into the same water from which they came. This is necessary to keep them alive until you get back to the laboratory. Fresh-water sponges are not easy to keep for long periods but can be kept in a balanced aquarium or out-of-door fish pool for a short time. They can stay there long enough for class use and study.

Preserving. Preservation of the fresh-water sponge is very simple. Simply allow the object to which the sponge is attached to dry and the sponge will dry also. Then it should be put into a mounting box because if it is touched it will crumble and fall apart. Preserving in a liquid, such as a five per cent formalin solution is adequate. How-

ever, if alcohol is easier to obtain a 70 per cent solution of alcohol may be used. (Miller and Blaydes, 1938)

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CHAPTER IV

FRESH-WATER FLATWORMS

Habitat. Planaria (Turbellaria) flatworms are found in most ponds and streams. They are found attached to various objects submerged and are usually on the under side of the object because they are strongly negative to light. The different species will have a corresponding difference in size and color. The direct range is from 1/8 inch to 1/2 inch and the color will vary from a white through brown or black. They are commonly dark gray.

Collecting. When visiting a pond or stream turn over sticks, stones, boards, etc., looking at the undersurfaces. Planarians are usually found in groups and clusters. They can be washed off an object into a collecting jar or can but if the container is too small you may transfer them with the point of a pinkknife. The collecting vessel should be closed until you reach the laboratory.

Another method for collecting is to tie a piece of meat, usually liver, to a string; drop it into a stream or pond for a few minutes and the planarians will attach themselves to the meat.

The planarians may be kept in an aquarium if stones or other objects are present on the bottom. Also, one must have a considerable amount of plants and dirt in the bottom of the

aquarium. Another method is to place the planerians in a porcelain pan with one inch of fresh water. Cover the pan to keep out the light. Feeding will be necessary and should be done two or three times a week. Small thin slices of liver are the best food for the planarians. The planarians will cluster upon it and feed. After an hour has passed the liver should be removed and the worms washed off the liver. A complete water change is best at this time to prevent fouling the culture. Worm cultures kept in this manner may run many years.

Preserving. The planarians are best preserved on microscope slides. Place the worm on a microslide and place another slide on top and apply pressure to flatten the worm but not so much as to crush it. Put the slide in a shallow dish and pour over it a solution of five per cent formalin. If the glass slides are tilted, the preservative will run between the slides and fix the worms. Allow them to stay in the solution for about two hours. Now remove the plates of glass and you will find the worms thin enough for microscope use. You may leave the worms between the slides until used, or remove the worms and place into vials. (Miller and Blaydes, 1938)

CHAPTER V

PARASITIC FLATWORMS AND ROUNDWORMS

Habitat. These worms are very widely distributed and their hosts include many of the other animals. However, some animals are likely to have more worms than others due to their eating habits. Some of the more common hosts are (1) the grass frog found in pools, ponds, and streams, (2) birds, especially chickens and fishing birds, (3) fish, those which prey upon other fish, (4) snails, (5) insects (but not common), (6) snakes and turtles, (7) rats, pigs, sheep, dogs, cats, and horses.

Collecting. Searching in cavities of the body which open to the outside will most likely contain parasites in the host. Amphibians, reptiles, and birds may have both flatworms and roundworms in the lung cavity. A hand lens or microscope will aid in finding them.

The stomach, small, and large intestines are likely spots for these worms. Sometimes they will be found encysted in the walls of these organs.

They are also found in the urinary bladder and sometimes in the liver and kidneys. Some forms may be taken from the blood stream but these are hard to find.

If a visit is made to the meat packing plant and fresh entrails of pigs and chickens are obtained, there should be

little difficulty in finding plenty of tapeworms and roundworms. This is almost a never failing source.

Preserving. First, one should kill the worms before preserving them. This may be accomplished in two ways:

(1) Put them in a pan or beaker of water and heat until the worms are limp and motionless. Then drop into a six or eight per cent formalin solution. (2) A small amount of grain alcohol could be added to some water containing worms. Then every few minutes add about the same amount of alcohol until the worms are anesthetized. Then preserve in formalin. (Miller and Blaydes, 1938)

CHAPTER VI

EARTHWORMS

Habitat. The earthworm (Annelida) is one of the most widely distributed animals and very easy to collect. They are best found where there is an abundance of decomposing vegetable matter and moisture in the soil. Using a shovel turn the soil in old manure piles or in heaps of dead leaves. This is likely to expose a few worms. Heavy rains, especially during spring or summer, will wash worms from elevated lawns out upon the sidewalks. A few minutes spent in collecting at such time may save hours of work later.

Collecting. With a flashlight and a can of dirt walk carefully over a freshly watered lawn at night in search of the worms. The worms lie stretched in the grass with posterior ends still in the burrows. Quickly grasp the worm and pull it out very slowly. A sudden jerk would probably break the worm into two parts, damaging the specimen.

After collecting, the worms should be kept in a considerable amount of soil containing humus and a layer of leaves over the top. The container should be placed in an area where the soil would be cool and damp. Feeding is necessary only when humus is not present in the soil.

Preserving. To preserve the earthworms so they will

be in excellent condition for later use, the following is a very good procedure: heat the worms in warm water as in killing, or stupefy them with alcohol until they become relaxed. Place the worm on a table and inject into the body cavity a six per cent formalin solution containing a little glycerin. Maintain the pressure until the segments become full. Inject about every ten segments until all segments are full. Then lay the worm in a pan and pour over it enough preservative to cover it. Let this stand overnight and the specimen will be in excellent condition for storage or dissecting. They can be stored in a jar or bottle containing a six per cent formalin solution. (Miller and Blaydes, 1938)

CHAPTER VII

MOLLUSKS

Habitat. Snails and clams are found in ponds and streams, while oysters may be purchased at markets. In a few areas the land snail is very abundant. Of course people living near the sea shore observe a variety of marine mollusks.

Clams are usually found in the muck or sand and in water but occasionally they are found in mud flats. Snails are found attached to growing plants and sticks or stones. The land forms are more likely found under stones, logs or in leaf mold on the ground.

Collecting. The mollusks may be collected along with other animals or plants. A jar consisting of a screw type lid or stopper is sufficient for a container. Snails can be gathered by hand. They should be placed in some water from which they are taken or the soil or leaf mold if you expect to keep them alive for any length of time. Clams can be removed from the muck and sand with a rake or hoe.

After collecting the snails, they can be kept for a long period just by placing them into an aquarium where plants are growing.

Clams are not as easy to keep but sometimes they live in an aquarium that has a lot of algae and other plants. Also,

the bottom should have several inches of muck, sand, and gravel.

Preserving. Snails can be killed if heated in water. They will protrude from their shells if done very slowly. Then they can be dropped directly into six or eight per cent formalin. The edge of the clam shell should be broken and a peg driven between the valves to allow formalin to penetrate. (Miller and Maydes, 1938)

CHAPTER VIII

CRUSTACEA

To collect aquatic crustaceans one should have various types of aquatic collecting equipment. Many can be collected with a dip net. A white enamel dipper is very good for collecting many of the smaller forms; the dipper is simply placed into the water and any small animal can be seen. These can be removed with an eye dipper or by forceps. Some of the smaller forms can be collected with a fine-mesh net called a plankton net. The shore-dwelling and terrestrial forms can be collected by hand or with forceps. The larger forms with well-developed claws, such as crayfish, should be picked up from above, grasping the animal at the back of the carapace.

One should collect in a variety of places to obtain various crustaceans. When collecting in water, be sure to investigate all the possible habitats. Some are free-swimmers, some burrow in the mud, some are found under stones, and many are found among aquatic vegetation.

Crustaceans should be preserved in fluids, usually a 70 to 95 per cent solution of alcohol. Most of the smaller forms must be mounted on microscope slides for detail study. (Borrer and DeLong, 1964)

CHAPTER IX

INSECTS

Habitat. Insects can be found nearly anywhere and the more places you look the more variety you are likely to collect. The best time to collect is in the summer but they can be found at most anytime between early spring to late fall and a few may be found in hibernation during the winter months. Most insects are active during the daytime hours, but some can be collected at any hour. Bad weather, such as rain or low temperature, will reduce the activity of most insects but others will not be affected and can be collected in any kind of weather. If one knows where to look he can find insects at any hour of the day and any day of the year.

Many kinds of insects feed on plants, therefore, plants are the best place for collecting. Insects can be swept off the plants with a net, picked, or shaken. Different insects feed on different plants, therefore, one should examine all kinds of plants, such as grasses, weeds, shrubs, and trees. Every part of the plant should be inspected but the majority will be on the leaves or flowers; others may be found on the stem, bark, wood, fruit or roots.

Various kinds of debris often harbor many kinds of

insects. Some may be found on leaf mold and litter on the top of the soil, especially in wooded areas where vegetation is abundant. Others can be found under stones, boards, bark, and similar objects; still others can be found in rotting material of all sorts such as fungi, decaying plants, or the bodies of dead animals, rotting fruits, and dung. Many can be picked up with the fingers or forceps and others can be taken by sifting debris.

In spring and summer during the evenings, insects from various places are attracted to lights and can be collected at street or porch lights, or at lights put up especially to attract them. In fact, this is one of the easiest ways to collect many types of insects. Blue lights seem to be more attractive to them than red or yellow.

Insects may be found in streams, lakes, or other bodies of water. Many aquatic insects can be collected by means of forceps; others are most easily collected by various types of aquatic collecting equipment. (Borror and DeLong, 1964)

Collecting. The collecting of insects is a very easy and simple matter. However, if they are to be kept as a permanent collection, they must be killed and mounted. With only a net and killing bottle many specimens may be collected.

Nets are usually of three types: (1) aerial nets for catching insects in flight, (2) sweeping or beating nets for taking insects hidden in vegetation, and (3) water nets for securing aquatic insects. All should be light but made of strong material. These may be bought from biological

supply houses or they may be made by hand.

Killing jars or bottles are very important and usually very dangerous if they aren't handled with care. Some compounds of cyanide make the best killing agent. Cyanide is a deadly poison. All cyanide bottles should be plainly marked and kept out of reach of small children. In fact, the cyanide killing jar is so dangerous that high school students should not be using it; therefore, it will not be discussed in detail.

A comparatively safe killing bottle may be made with ethyl acetate. Fill the bottom half inch to an inch of the bottle with plaster of paris. When completely dry, saturate the plaster with ethyl acetate, pouring off any excess liquid. Killing bottles or jars must be kept tightly covered with a cork or lid but will last for months and may be revived again by drying the plaster and recharging. Insect specimens do not become brittle as soon in bottles of this type as in cyanide killing bottles or jars.

Other killing agents such as chloroform, ether, gasoline, benzine, and carbon tetrachloride may be used by collectors.

Many kinds of small insects may be put into vials of 80 per cent alcohol for killing and preserving.

Killing bottles should be about half or more filled with loose bits of soft paper. These keep insects from damaging each other through their movements before they are dead and help keep the bottles dry. When the catch is emptied, these folded papers, if damp, should be destroyed

and fresh ones put in their place.

One should have special bottles for butterflies and moths and do not put other insects into them. The scales from the wings of moths and butterflies come off and spoil specimens of other orders. Occasionally wipe out these bottles to remove the loose scales. Another method is to kill the moth or butterfly by squeezing the body, which prevents the loss of scales.

One should have one or more bottles for bees and flies, and not put anything else into them. Remove flies and bees soon after they are dead and pack them lightly in soft paper, in small cardboard boxes. It is best to remove all specimens from killing bottles and pack them between layers of cotton wadding. Insects marked with yellow often turn red or orange if left too long in a cyanide bottle. Even the most sturdily built specimens should not stay over 24 hours in a killing bottle. (Jaques, 1947)

Preserving. Insects can be mounted and preserved in many ways. Most specimens are pinned and after they have dried will usually keep indefinitely. Insects too small to pin can be mounted on "points", on very small pins, or on a microscope slide. Showy insects, such as butterflies, moths, grasshoppers, dragonflies or damselflies, may be mounted in various types of glass-topped display cases. Soft-bodied forms will probably do better in small vials containing a preserving fluid.

Pinning is the best way to preserve large hard-bodied

insects; pinned individuals retain normal appearance, and are easily handled and studied. The colors usually fade when the insect dries, but this is very difficult to avoid. Bright colors are generally better preserved if the specimens are dried very fast.

Common pins aren't very good for pinning insects; they are usually too thick and too short, and they rust. Insects should be typed with a special pin made of steel and known as an insect pin. These pins are longer than common pins and come in various thicknesses ranging from 00 to 7 respectively. Smaller sizes are best for general use, such as sizes two and three. These may be purchased from various supply houses.

Insects are usually pinned vertically through the body. Forms such as bees, wasps, flies, butterflies and moths are pinned through the thorax between the base of the front wing. Bugs are usually pinned through the scutellum, a little to the right of the midline. Grasshoppers are pinned through the posterior part of the pronotum, a little to the right of the midline. Beetles should be pinned through the right elytron about halfway between the two ends of the body.

The easiest way to pin an insect is to hold it between the thumb and forefinger of one hand and insert the pin with the other. All should be mounted at the same height about $\frac{1}{2}$ inch from the top of the pin. All should have dorsal side up and facing forward.

Insects too small to pin may be mounted on a card point.

Points are elongated triangular pieces of light cardboard or celluloid; the point is pinned through the base, and the insect is glued to the tip of the point.

Putting specimens on a point is a very simple process. The point is put on the pin at the base; the pin is grasped by the pointed end and the upper side of the point is dipped into the glue and then attached to the insect. A very little glue is all that is necessary. The insect is mounted dorsal side up and the head should be pointing forward.

The glue used for mounting insects on points should be quick-drying and quite hard when it dries. Any type of commercial glue is suitable. (Borror and DeLong, 1964)

Butterflies and moths must be dried with the wings spread and pinned. Special spreading boards can be bought but a substitute can be made by cutting slits in a cardboard box. Slits of various sizes can be made to fit the bodies of the insects, and the wings are spread at the sides. They should be put away to dry for at least a week before placing into a collection box.

Dragonflies and damselflies often lose wings and bodies when kept on pins; therefore, it is sometimes best to keep them in plastic or cellophane envelopes.

Putting insects of any kind into a vial or small jar containing a 70 per cent solution of alcohol is a very good preservative.

Insect labels of very small size are placed on the pin below the specimen. These include locality, date and name

of collector. A second label giving the name of the insect may be added later. Other data, such as host plant of the insect and habitat, may be added as it is needed. (Benton and Werner, Jr., 1957)

SUMMARY

It is the hope of the author that the material listed above will be beneficial to the teacher of the biological sciences. The aim is to give the teacher some knowledge for collecting, culturing, and preserving invertebrates. This will provide living material for the laboratory and a preserved stock for future use. The following is a summary of organisms mentioned and characteristics for each.

Protozoa: The protozoa are one-celled animals found most generally in aquatic environments. They may be collected in jars or bottles when collecting from a pond. If they are being cultured from dead grass or leaves most any kind of container will be suitable. To preserve the protozoans a ten per cent solution of formalin is very good and will preserve most forms.

Fresh-Water Sponges: These are all found in aquatic habitats. Even though some are marine the ones discussed are all found in fresh-water. Collecting can be done with a can or jar but keep them in the same water from which they came. Preserving is very simple; just allow the sponge to dry on the object to which it is attached or put them into a five per cent solution of formalin.

Fresh-Water Flatworms: Flatworms are in most aquatic areas attached to various objects. Collecting is very simple, just turn over stones and other debris. Planarians, the most common type of fresh-water flatworms, are best preserved on

a microscope slide.

Parasitic Flatworms and Roundworms: These worms are widely distributed in host of many aquatic and terrestrial animals. Usually the host is collected and then dissected to locate the worms. They can be preserved in a six or eight per cent solution of formalin.

Earthworms: These are found in the soil containing decomposing vegetation. They may be collected by digging and searching in this type of soil. A six per cent formalin solution is injected about every ten segments for preservation.

Mollusks: Snails and clams are the most common forms for this group and they can be found in ponds and streams. Snails can be collected by hand while the clams are taken from the muck and mud with a rake or hoe. They are preserved in a six or eight per cent solution of formalin.

Insects: These are the largest group of invertebrates and as you might expect are found in all conceivable places. Insects are collected with all kinds of collecting equipment, such as nets, killing jars, traps, etc. . They are usually preserved according to their size. The small ones are put on microscope slides or in vials containing alcohol. The larger ones will be pinned or placed into specially folded paper.

Crustaceans: Crustaceans are both aquatic and terrestrial. The aquatic forms are collected with various types of aquatic collecting equipment. The terrestrial forms can be collected with the hands or forceps. Preservation is best in a 70 to 95 per cent solution of alcohol.

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