Spicule preparations
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There are several ways to prepare microscopic slides necessary for taxonomic identification. Two are in general use and relatively simple: spicule preparations (see below) and hand sections (see "Thick sections").

Spicule preparations:
Two methods are here described, bleach and nitric acid preparations.
A. Bleach preparation:
This is a "quick and dirty" method, suitable for quick identifications of all spicule bearing sponges including Calcarea.
You will need:
-small plastic containers like eppendorfs
-undiluted domestic bleach
-96% ethanol in squeeze bottle
-distilled water in squeeze bottle
-razor blades or scalpels
-petri dish for cutting sponge
-disposable pipettes

1. Cut a small portion of ectosome and choanosome (3 cubic mm) on petri dish, and place in eppendorf (rinse off utensils between specimens). Label lid eppendorf with specimen number.
2. Half fill each eppendorf with bleach and let sit for 30-60 minutes depending on the consistency of the sponge.
NOTE: If you don't need a permanent mount of your spicules, you can look at the spicule- bleach suspension pipetted out of the eppendorf and spread on a slide topped with a coverslip. The slide can then be washed and re-used.
3. Pipette off as much bleach carefully as possible without disturbing dissolved tissue and spicules.
4. Squirt in water to carefully resuspend spicules; let spicules settle over 10-15 minutes; repeat several times.
5. Pipette off water as in step 3 and refill with 100% ethanol to resuspend spicules.
6. Aspirate spicule suspension into pipette.
7. Gently squirt onto warming slide, spread around a little with pipette tip, dry off ethanol, add mounting medium, and add a coverslip.

B. Nitric acid spicule preparations:
This technique will give you very clean spicules that are suitable for the examination ofmicroscleres and scanning electron microscopy. It is not suitable for Calcarea.
You will need:
-nitric acid * (HNO_3) in glass
-ddH20 in squeeze bottle
-96% ethanol in squeeze bottle
-plastic centrifugetubes
-50ml Erlenmeyer flasks
-petri dishes
-razor blades or scalpels
-disposable pipettes
-slides, coverslips
-desk-top centrifuge

* CAUTION: HIGHLY CORROSIVE, DO NOT INHALE FUMES, WEAR GLOVES,
LABCOAT, SAFETY GLASSES. Do as many manipulations as possible with nitric acid under a fume hood.

1. Cut a small portion of ectosome and choanosome (3 cubic mm) on a petri dish, and place in an erlenmeyer flask or centrifugetube (rinse off utensils between specimens to avoid cross- contamination from other species)
2. UNDER FUME HOOD AND VERY, VERY CAREFULLY (WITH GLOVES, LAB COAT, AND SAFETY GLASSES), pour nitric acid into flasks or centrifugetubes to just cover sponge (not too much or it will bubble over

and won't fit in centrifugetube), allow to sit for as long as it takes to dissolve all tissue (over lunch) or heat gently over a Bunsen flame, heating block, or microwave (5-10 seconds). Swirl contents until all brown gas is boiled off and liquid is clear, pale yellow. Hold mouth of flask or centrifugetube away from you with claspers.
3. Pour contents of each flask into the appropriately labelled centrifugetube (if you are already using centrifugetube you are already OK), line all up in rack, balance with ddH20 by eye, place in centrifuge, spin 3-5 min at 3000 rpm.
4. Tip off nitric acid into a plastic container in sink being constantly filled with tap water (to immediately dilute acid). Do this with a quick action so as not to disturb pellet.
5. Fill centrifugetube half way with ddH2O dislodging pellet. If you can't dislodge pellet, cap and shake. Balance all centrifugetubes with water and spin as in step 3.
6. Tip off water as in step 4, squirt in ethanol as in step 5, spin.
7. During this spin turn on slide heater tray, have slides cleaned, labelled, and heating on tray.
8. Pour off ethanol, trying to retain a little to flick and resuspend the pellet. If you can't do this add a small squirt of ethanol to dilute spicules.
9. Pipette spicules on to warming slides, changing pipettes in between specimens (or wash in a large beaker), allow to dry, don't sneeze!
10. Lay coverslips out on a clean sheet of paper, pipette an appropriate amount of mountant onto slip one at a time, overturn slide and gently lower onto coverslip FROM ONE SIDE (TO AVOID BUBBLES)- Quickly turn the slide and c-slip right-side-up and allow to dry overnight at room temp (drying ovens make the mountant shrink).
For SEM preparations similar procedures (except for step 10) can be employed.

Source: Kelly-Borges and Pomponi, 1992.