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Measurement of small tissue volumes using Holden's apparatus

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Abstract

A reproducible method for the estimation of volumes of small tissue samples was needed to allow comparison of the biological activity of tissue explants and to relate symptom improvement to procedures such as adenoidectomy or polypectomy. Holden's apparatus, which utilizes fluid displacement between two chambers, was compared to displacement in a 20 ml syringe. Two observers made four readings each on 20 tissue samples (range 0.37 to 4.70 ml) using both methods for comparison.

A two-way analysis of variance for replicated data gave an F ratio of 0.6885 (df = 19; $p>0.8$) for Holden's apparatus, and an F ratio of 1.4307 (df = 19; $p>0.1$) for the 20 ml syringe. The standard error of the mean for repeated readings on the same sample by an observer was 0.001 ml for Holden's apparatus and 0.014 ml for the syringe method. For accurate estimation of small tissue volumes within 0.01 ml we recommend Holden's apparatus.

Key words: Tissue, volume estimation

Introduction

Measurement of small tissue volumes removed during otorhinolaryngological procedures is useful for comparing biological activity (Mason *et al.*, 1994) of the tissue and relating symptomatic improvement to volume of tissue removed, as in turbinectomy, adenoidectomy or polypectomy (O'Flynn, 1993).

Measurement of tissue volumes is most accurately carried out by a displacement method in a chamber of sufficient dimensions not to compress the tissue. Volume changes are estimated from a graduated scale on the side of the chamber. Determination of volume of small pieces of tissue by immersion in a 20 ml syringe are prone to error because a 1.0 ml displacement is represented by a 3.5 mm change in the meniscus level, and there are no graduations of less than 1.0 ml. We describe in detail Holden's apparatus which utilizes the principle described by Aherne and Dunhill (1982) whereby the tissue is placed in a large chamber that does not compress the tissue, but the displacement of the meniscus level is read in a 10 ml pipette with 0.1 ml gradations that are nearly 2 mm apart and a fine bore 1.0 ml pipette with gradations of 0.01 ml that are over 1 mm apart. This was compared to the method of displacement in a single chamber graduated 20 ml syringe (O'Flynn, 1993).

Materials and methods

Measuring tissue volume using Holden's apparatus

A universal container had a hole fashioned into its lid with a soldering iron and a graduated 1.0 ml pipette with 0.01 ml gradations was fitted flush with the lid and secured with hot melt adhesive (RS components). The lid was secured with finger

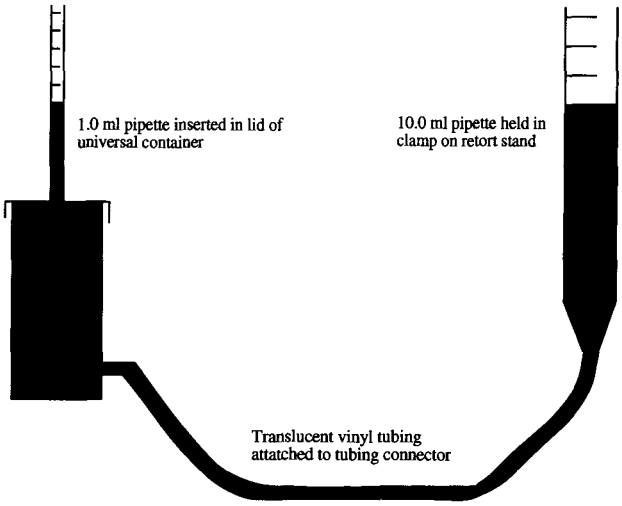

FIG. 1

Holden's apparatus for measuring small tissue sample volumes.

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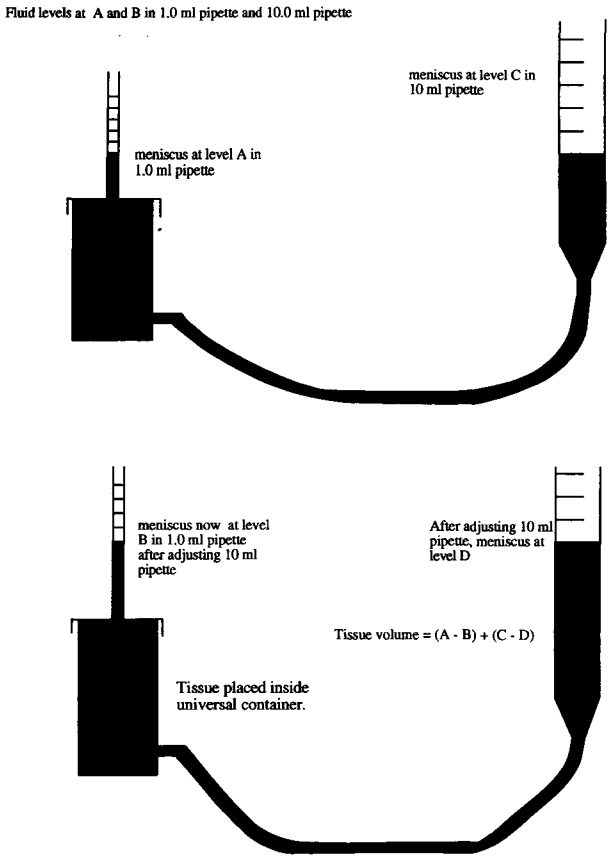

FIG. 2
Method of measuring tissue volumes using Holden's apparatus.

tightness so that the gradations faced forwards. Just above the base of the universal container a second hole was made similarly and an '8 to 12' mm tubing connector (Portex) was placed into the hole and secured with hot melt glue. Translucent vinyl tubing (8 mm; Portex) connected the universal container to a 10 ml pipette, with 0.1 ml gradations (Figure 1). The universal container was held vertically at a fixed point on a retort stand, and the 10 ml pipette was held in a clamp in a vertical position but could be moved up and down the retort stand.

The universal container, tubing and 10 ml pipette were filled with minimal essential medium (MEM; Flo laboratories) ensuring that there was no air in the system. MEM is pink, aiding visualization of the meniscus, and as an isotonic culture medium it maintains the tissue. The height of the 10 ml pipette was raised so the MEM rose into the 1 ml pipette, the height of the 10 ml pipette was adjusted so that the meniscus was exactly on an 'n.0 or n.5 ml' gradation. The meniscus levels in the two pipettes were noted. The 10 ml pipette was lowered so the level of the MEM dropped below the lid of the universal container. The lid was removed and the tissue placed inside. On replacing the lid it was tightened with finger tightness so that the scale of the 1 ml pipette faced forwards. The 10 ml pipette was then raised until the meniscus in this pipette was exactly on an 'n.0 or n.5 ml' gradation. The new meniscus levels in each pipette were noted, and the volume of the tissue sample calculated as the sum of the differences between the two levels (Figure 2).

Measurement of tissue volume using the displacement method

A 20 ml syringe with 1 ml gradations had its nozzle capped off and plunger removed. Fluid was placed in the syringe so that the meniscus was exactly on an 'n.0 ml' gradation, the tissue was then completely immersed in the fluid and the volume was estimated from the displacement of the meniscus level.

Comparison of the two methods of volume estimation

Two observers performed independent blind estimations of tissue volumes on 20 different tissue

TABLE I
PART I: ESTIMATED VOLUMES FOR EACH TISSUE SAMPLE BY EACH OBSERVER (IN THIS CASE OBSERVER 1) USING THE TWO DIFFERENT METHODS

	20 ml Syringe displacement				Holden's apparatus			
Observer 1	1	2	3	4	1	2	3	4
Sample 1	0.8	0.7	0.7	0.6	0.84	0.83	0.85	0.84
Sample 2	2.8	2.7	2.9	2.9	2.73	2.72	2.71	2.73
Sample 3	4.5	4.6	4.8	4.8	4.64	4.63	4.63	4.63
Sample 4	2.4	2.5	2.6	2.4	2.47	2.46	2.48	2.47
Sample 5	4.3	4.3	4.4	4.6	4.42	4.42	4.42	4.43
Sample 6	0.4	0.5	0.5	0.5	0.51	0.50	0.50	0.51
Sample 7	0.2	0.3	0.3	0.4	0.36	0.37	0.36	0.36
Sample 8	1.7	1.5	1.4	1.5	1.44	1.43	1.44	1.44
Sample 9	0.4	0.5	0.6	0.5	0.64	0.62	0.62	0.62
Sample 10	2.3	2.6	2.4	2.5	2.60	2.63	2.62	2.62
Sample 11	1.4	1.3	1.4	1.2	1.26	1.28	1.29	1.27
Sample 12	3.3	3.4	3.5	3.6	3.39	3.40	3.38	3.39
Sample 13	3.0	3.2	3.1	3.0	3.11	3.13	3.12	3.12
Sample 14	2.5	2.5	2.6	2.7	2.55	2.55	2.56	2.55
Sample 15	1.6	1.4	1.5	1.6	1.65	1.66	1.67	1.67
Sample 16	0.9	1.1	1.2	0.9	0.98	0.97	0.99	1.00
Sample 17	2.1	2.3	2.4	2.5	2.23	2.24	2.25	2.24
Sample 18	3.0	3.1	3.3	3.3	3.09	3.10	3.11	3.10
Sample 19	0.4	0.5	0.6	0.5	0.43	0.44	0.44	0.45
Sample 20	1.5	1.5	1.4	1.5	1.34	1.35	1.33	1.33

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TABLE I

PART 2: ESTIMATED VOLUMES FOR EACH TISSUE SAMPLE BY EACH OBSERVER (IN THIS CASE OBSERVER 2) USING THE TWO DIFFERENT METHODS

	20 ml Syringe displacement				Holden's apparatus			
Observer 2	1	2	3	4	1	2	3	4
Sample 1	0.6	0.7	0.8	0.9	0.83	0.83	0.83	0.84
Sample 2	2.9	3.0	3.0	2.9	2.71	2.71	2.73	2.71
Sample 3	4.7	4.7	4.8	4.9	4.63	4.63	4.63	4.62
Sample 4	2.6	2.4	2.5	2.4	2.48	2.47	2.46	2.46
Sample 5	4.6	4.7	4.5	4.7	4.43	4.43	4.42	4.42
Sample 6	0.4	0.5	0.6	0.7	0.51	0.51	0.52	0.50
Sample 7	0.3	0.3	0.4	0.5	0.37	0.37	0.37	0.36
Sample 8	1.3	1.5	1.4	1.3	1.43	1.44	1.43	1.44
Sample 9	0.3	0.6	0.6	0.4	0.61	0.63	0.63	0.63
Sample 10	2.3	2.3	2.4	2.5	2.63	2.61	2.61	2.62
Sample 11	1.2	1.4	1.6	1.7	1.29	1.28	1.26	1.28
Sample 12	3.1	3.2	3.4	3.4	3.37	3.39	3.39	3.39
Sample 13	3.0	3.0	3.1	3.1	3.11	3.13	3.11	3.12
Sample 14	2.4	2.4	2.6	2.6	2.56	2.57	2.58	2.55
Sample 15	1.7	1.4	1.4	1.3	1.65	1.66	1.67	1.67
Sample 16	0.8	1.1	1.2	0.8	0.97	0.98	0.99	0.99
Sample 17	2.3	2.4	2.3	2.1	2.22	2.24	2.24	2.25
Sample 18	3.0	3.1	3.0	3.3	3.09	3.11	3.11	3.09
Sample 19	0.4	0.3	0.6	0.5	0.43	0.43	0.45	0.43
Sample 20	1.4	1.5	1.2	1.5	1.33	1.34	1.33	1.35

samples using inferior turbinates, nasal polyps, adenoids and tonsils. Each made four separate estimations of the volume of the tissue using the two different methods (see Table I).

Results

The range of volumes measured was 0.37 to 4.70 ml. Using Holden's apparatus it was possible to measure volumes to the nearest 0.01 ml, but with the 20 ml syringe the volumes were estimated to the nearest 0.1 ml.

Using Holden's apparatus the mean volume for both observers was 2.036 ml and the standard error for repeated measurements by either observer on the same piece of tissue was 0.00102 ml. For the syringe method the mean volumes were 2.04 and 2.03 ml and the standard error for repeated measurements by either observer was 0.014 ml. For Holden's apparatus two-way analysis of variance for replicated data gave an F ratio of 0.6885 (df = 19; $p>0.8$) whereas for the syringe method the F ratio was 1.4307 (df = 19; $p>0.1$).

Discussion

The interaction between tissue and observer did not contribute significantly to the variance in volume measurement, when four repeated measurements were used, and estimates were to 0.01 ml for Holden's apparatus and 0.1 ml for syringe displacement. When measuring tissue volumes below 1.0 ml this represents a potential error of one or 10 per cent respectively.

Conclusion

Holden's apparatus is cheap and easy to construct. It is portable and straightforward to use, and accurate when measuring volumes below 1.0 ml. For accurate estimation of small tissue volumes within 0.01 ml we recommend Holden's apparatus.

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