

# COMPARING METHODS OF DETERMINING FORMALIN CONCENTRATION IN FLUID PRESERVATIVES

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**Abstract.**—Formalin (3.7% weight/weight [w/w], 4% weight/volume [w/v] aqueous formaldehyde) is commonly used as a fixative to prevent postmortem changes in the tissues of a specimen. The specimen is then either maintained in formalin or transferred to another fluid preservative, such as ethanol or isopropanol, for long-term preservation. Residual formalin often remains in the preservation fluid. As formaldehyde is a known carcinogen, it is important to know the concentration present in preservation fluids.

A titration method has been developed to determine formalin concentration in fluid preservatives. It utilizes sodium sulfite to react with formaldehyde in solution. An adjustable, repeating pipette and a digital titrator allow for the rapid determination of formalin concentrations in small samples (<1 ml). This method of titration is compared with three commercially available methods of determining formaldehyde and formalin concentration: two brands of formaldehyde test strips, Quantofix<sup>®</sup> (Machery-Nagel GmbH) and MQuant<sup>®</sup> (EMD Millipore Corp), and a drop count titration test kit (Hach<sup>®</sup> formaldehyde test kit, model FM-1). A comparison and evaluation are made on the effectiveness of each method in determining the concentration of formalin in preservation fluids.

**Résumé.**—Le formol (solution aqueuse à 3,7% w/w ou 4% w/v de formaldéhyde aqueux) est couramment utilisée comme fixateur pour prévenir les changements post mortem dans les tissus d'un spécimen. Le spécimen est ensuite soit maintenu dans le formol, soit transféré dans un autre liquide de conservation, comme l'éthanol ou l'isopropanol, pour une conservation à long terme. Le formol résiduel reste souvent dans le liquide de conservation. Comme le formaldéhyde est un cancérigène connu, il est important de connaître la concentration présente dans les liquides de conservation.

Une méthode de titrage a été mise au point pour déterminer et calculer la concentration de formol dans les liquides de conservation. Elle utilise du sulfite de sodium qui réagit avec le formaldéhyde en solution. Une pipette automatique réglable et un titrateur numérique permettent de déterminer rapidement les concentrations de formol dans de petits échantillons (<1 mL).

Cette méthode de titrage est comparée à trois méthodes disponibles dans le commerce pour déterminer la concentration de formaldéhyde: des bandelettes de test pour le formaldéhyde, Quantofix<sup>®</sup> (Machery-Nagel GmbH) et MQuant<sup>®</sup> (EMD Millipore Corp); et un kit de titrage par compte-gouttes (Hach<sup>®</sup> Formaldehyde Test Kit - Model FM-1). Une comparaison et une évaluation de l'efficacité sont proposées pour chaque méthode de détermination de la concentration de formaldéhyde dans les fluides de conservation.

**Key words.**—concentration, fluid preservatives, formaldehyde, formalin, test strips, titration.

## INTRODUCTION

It has long been possible to determine the concentration of alcohol-water fluid preservative solutions through density measurement with a hydrometer or digital density meter. These have given collection managers the ability to rapidly and accurately determine the concentration of alcohol in even very small volumes of fluid preservative and in the presence of moderate concentrations of additives (Boase and Waller 1994). Formalin is commonly used as a fixative for fluid-preserved specimens and sometimes as a long-term preservative. Residual formalin from fixation often remains in alcohol-based fluid preservatives. A means

of determining the presence of formalin in preservative solutions (Waller and McAllister 1987) has been available for more than 30 years.

Quantitative determinations of formalin concentrations in fluid preservatives are still not generally performed, and their use has been restricted to specific studies. Methods to determine if aldehydes are present in fluid preservatives include the use of Schiff's reagent (Moore 2009) and commercial formaldehyde test strips. Kay and Ivison (2007) and Van Dam (2009) utilized semiquantitative commercial formaldehyde test strips to determine formalin concentration in fluid preservatives. While these test strips state that they are for determining formaldehyde concentration, other aldehydes contribute to results (Machery-Nagel 2013, Merck 2018) as they do for titrations. Titration methods to determine formalin concentration have been used by Simmons and Waller (1994), Waller and McAllister (1987), and Waller and Simmons (2003).

The limited studies into formalin concentration in fluid preservatives are a result of the fact that reliable determinations of formalin or formaldehyde concentration often require analysis using more complex instrumental methods or titration. Titration, while not complex, does require the use of equipment and skills not commonly available in natural history museums. The sodium sulfite method of determining formalin concentration described in this paper takes advantage of laboratory-scale fluid-dispensing equipment to provide a moderate-cost setup for the rapid determination of formalin concentrations in small samples.

This paper compares the sodium sulfite titration method, initially published by Walker (1964) and adapted by Waller (unpubl. ms. 1993) for tests on fluid preservatives, with three commercially available methods to determine formalin concentration in fluid preservatives. Formaldehyde is a known carcinogen (Babin et al. 2010, IARC 2018, Liteplo et al. 2002, Simmons 2014), so for health and safety reasons, or for fluid transfers, it is important to know the concentration of formaldehyde in solution, be it present as an ongoing preservative or residual formalin from fixation. Testing these different methods enabled assessment of which methods were the simplest to use and gave the most accurate results.

### *Terminology*

There is often confusion surrounding the use of the terms formalin and formaldehyde in reference to their concentrations in fluid preservatives. As described in Simmons (2014), the term formalin may refer to a saturated solution of formaldehyde gas in water or any dilution of that saturated solution. A saturated solution of formaldehyde gas in water, at room temperature, is 37% weight/weight (w/w) or 40% weight/volume (w/v) formaldehyde gas in solution, commonly referred to as 100% formalin stock solution. In both expressions, the quantity of formaldehyde is expressed by weight, while the quantity of the solution may be expressed by weight for w/w or by volume for w/v. This 100% stock solution is then diluted in a ratio of one part stock solution with nine parts water to make what is commonly referred to as 10% formalin. The 10% volume/volume (v/v) formalin therefore contains 3.7% w/w or 4% w/v formaldehyde gas in water, equivalent to 40 g/L formaldehyde.

### METHODS

Four methods to determine formalin concentration in fluid preservatives were compared. These included two sodium sulfite titration methods (a digital titrator method and a commercially available formaldehyde test kit) and two commercially available formaldehyde test strips, which provide semiquantitative results.

All tests were conducted within a fume hood to minimize exposure to vapors from fluid preservatives. Appropriate personal protective equipment, including a lab coat, nitrile gloves, and protective eye wear, were worn (Hawks et al. 2010; Simmons 2014, 2019).

### *Sodium Sulfite Titration Method*

This method utilizes titration to determine the amount of formaldehyde in solution. Thymolphthalein is used as the pH indicator. The sample, along with pH-adjusted water, is initially titrated to the transition point of thymolphthalein (light blue color) to remove any titratable acidity that may affect the titration results. Sodium sulfite is added and reacts with formaldehyde, releasing hydroxide as part of the reaction. As a highly alkaline solution has been formed, the fluid changes to a darker blue tone due to the thymolphthalein pH indicator. The sample is then titrated with hydrochloric acid back to the initial light blue tone. The amount of hydrochloric acid required to return the fluid to the light blue tone indicates how much hydroxide formed when the sodium sulfite was added, and therefore how much formaldehyde was initially in the sample.

### *Analytical Method*

The method of analysis employed is the convenient and accurate sulfite method (Walker 1964). The sulfite method relies on titration of hydroxide released when a solution of sodium sulfite reacts with a formalin solution to form a formaldehyde-sulfite addition compound and free hydroxide according to reaction 1:



For accurate results, it is essential that the sample is free of titratable acidity. Rather than being a hindrance, this provides an opportunity to determine titratable acidity as a step within the formalin concentration determination.

Two pieces of equipment that allow for a simple and rapid procedure to be established are an adjustable, repeating pipette and a digital titrator with calibrated titrant solution in a prepackaged and ready-for-use container. Two titrators are preferred for speed and precision (via back titrating with one-fifth strength titrant) and also for determination of titratable acidity with the same apparatus.

Using an adjustable repeating pipette allows a sample volume to be selected so that the digital titrator will read the formalin concentration directly. The procedure described here is designed so that the titrator will read 100 for a 10% formalin solution. By marking a decimal between the first and second digits of the titrator, it will read formalin concentration directly to 0.1% formalin.

To determine the sample volume needed for the titrator to read directly in percent formalin, both the stoichiometry of reaction (1) and the formaldehyde concentration in formalin solutions must be considered. The stoichiometry of the formaldehyde-sulfite reaction dictates that

$$F = C \times V \times M$$

where  $F$  is mass of formaldehyde in the tested solution in g;  $C$  is the concentration of the titrating solution in moles/liter (mol/L);  $V$  is the volume of titrating solution added in L; and  $M$  is the molecular weight of formaldehyde (30.03 g/mol).

In this case, the concentration of hydrochloric acid titrant used is 8.00 ( $\pm 0.04$ ) (mol/L). The titrator delivers 0.125 ml per 100 digits. Consequently, the amount of formaldehyde

required in the sample to give a result of 100 on the titrator is

$$F = 8.00 \text{ mol/L} \times (0.125 \times 10^{-3} \text{ L}) \times 30.03 \text{ g/mol} \\ = 0.03003 \text{ g}$$

The concentration of 100% formalin is taken to be 37% w/w, which is 40% w/v or 400 g/L. Hence, the concentration of a 10% solution of formalin is 40 g/L formaldehyde. Thus, the volume of 10% formalin containing 0.03003 g formaldehyde is then

$$\text{Volume} = 0.03003 \text{ g}/40 \text{ g/L} = 0.75 \text{ ml}$$

Consequently, by using a 0.75 ml sample the titrator will indicate percent formalin  $\times$  10. This is a convenient sample size in that it can be measured with reasonable accuracy with an adjustable repeating pipette ( $\pm 2\%$ ) and allows sampling from containers holding small volumes of fluid preservative. It is, however, too small a volume to easily titrate. This problem is overcome by adding 10 ml of deionized water pH adjusted to the indicator endpoint.

The quantity of sodium sulfite required to react with the maximum anticipated formalin concentration can also be calculated by considering the stoichiometry of reaction (1). A convenient volume of sodium sulfite solution to use is 10 ml. If the concentration of this solution is set at 0.5 mol/L, then the solution could react with 0.15 g formaldehyde, equivalent to a 50% formalin solution. This is thought to be sufficient. In the rare (and not yet encountered) event that a result of 45% formalin or higher is obtained, the titration should be repeated using a larger quantity of sodium sulfite solution.

### *Equipment and Materials*

The following equipment and expendable materials are required to undertake the sodium sulfite titration method, set up as shown in Figure 1. Many institutions will already have some of the equipment and supplies available, and this will significantly reduce the setup costs. Some of the equipment is available from numerous suppliers, and indication here of manufacturers or suppliers is not an endorsement.

#### Equipment:

- Adjustable pipette (100–1,000  $\mu\text{l}$ ) (Fisher Scientific, Fisherbrand™ Elite™ adjustable-volume pipetter, catalog no. FBE01000) with 1,000  $\mu\text{l}$  pipette tips (Fisher Scientific, World Precision Instrument 100-1,000  $\mu\text{l}$ , catalog no. 50-822-082).
- Two digital titrators, 0.00125 ml/digit, one fitted with a straight delivery tube (Hach® Company, digital titrator, product no. 1690001), the other fitted with a 90° delivery tube (Hach Company, product no. 4157800).
- Magnetic stirrer (Thermo Scientific™ RT Basic Series magnetic stirrer, catalog no. 90-691-18) and magnetic stir bars (Chemglass Life Sciences, item no. GC-2005-10).
- Support stand (Hach Company, ring stand support, product no. 56300).
- Two adjustable support clamps (Fisher Scientific, Fisherbrand™ Castaloy™ three-prong extension clamps, catalog no. 05-769-8Q) and one all position clamp holder (Fisher Scientific, Troemner™ Talboys™ all-position clamp holder, catalog no. 02-300-222).
- Multiple 25 ml Erlenmeyer flasks (Fisher Scientific, Fisherbrand reusable glass narrow-mouth Erlenmeyer flasks, 12 pack, catalog no. FB50025).



Figure 1. Setup of equipment for sodium sulfite titration method. (© I. Finkelde.)

- Two 10 ml graduated cylinders (Fisher Scientific, Pyrex™ single metric scale graduated cylinder with funnel top, catalog no. S14281).
- Two 250 ml glass bottles with good closures (Sigma-Aldrich, Pyrex® round media storage bottles and reusable screw caps, catalog no. CLS1395250-1EA).
- Two 50 ml beakers (any laboratory equipment supplier).
- Wash bottle with deionized or reverse osmosis (RO) water (any laboratory equipment supplier).
- Two plastic (high-density polyethylene) bottles to store delivery tubes (any laboratory equipment supplier).

Expendable materials:

- 8.0 mol/L hydrochloric acid (HCl) titrant cartridges (Hach Company, product no. 1439001).
- 1.6 mol/L sodium hydroxide (NaOH) titrant cartridges (Hach Company, product no. 1437901).
- Thymolphthalein indicator solution (Fisher Scientific, LabChem™ thymolphthalein, 0.1% in ethanol, 125 ml, catalog no. LC260757).
- Sodium sulfite, anhydrous (Sigma-Aldrich EMSURE® Reag. Ph Eur 500 g, catalog no. 1066570500).
- Deionized or RO water (any laboratory equipment supplier).

The detailed protocol for the sodium sulfite titration can be found in Appendix 1. Refer to Figure 2 for an indication of the color shifts that occur during the titration. The darkness of the blue tone that develops when the sodium sulfite is added corresponds to the amount of formalin present in the sample (Figure 3).

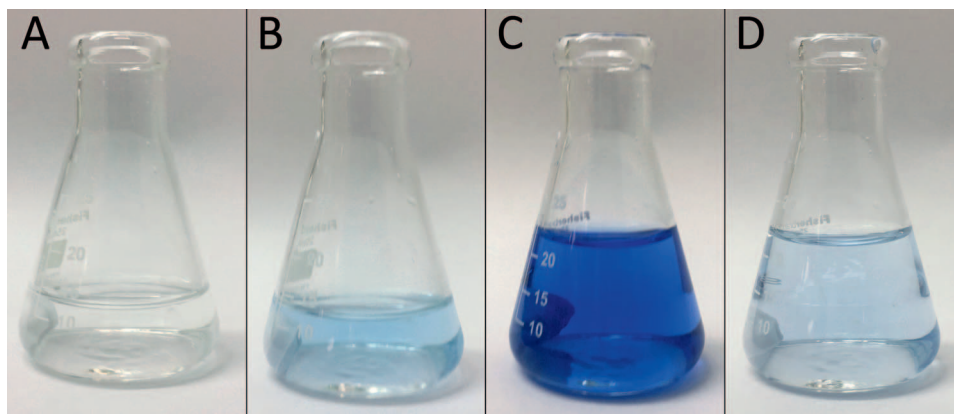


Figure 2. Sodium sulfite titration method: (A) 750  $\mu$ l sample of 10% v/v NBF with 10 ml pH-adjusted water and three drops thymolphthalein indicator. (B) 10% v/v NBF sample titrated with 10 digits 1.6 mol/L NaOH. (C) 10% v/v NBF sample with 10 ml 0.5 mol/L  $\text{Na}_2\text{SO}_3$  solution added. (D) 10% v/v NBF sample titrated with 100 digits 8.0 mol/L HCl, for a result of 10.0% v/v formalin. (© I. Finkelde.)

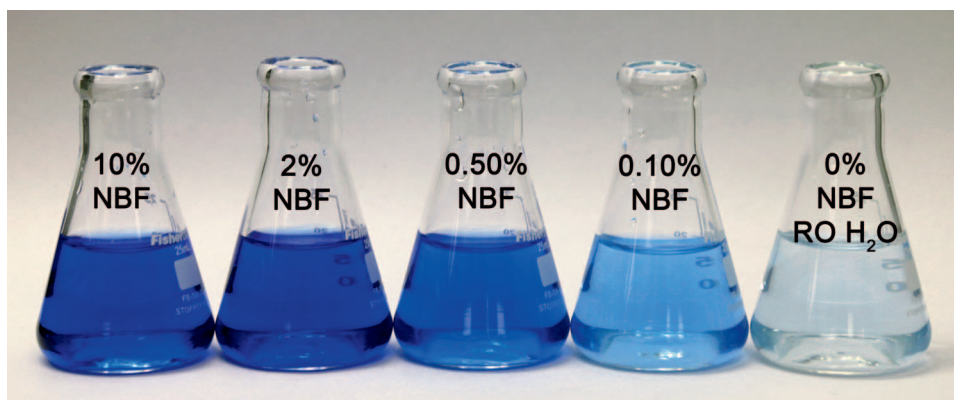


Figure 3. The darkness of the blue tone that develops when the 10 ml 0.5 mol/L  $\text{Na}_2\text{SO}_3$  solution is added to the sample corresponds with the amount of formalin in the sample. Left to right: 10% v/v NBF; 2% v/v NBF; 0.50% v/v NBF; 0.10% v/v NBF; 0% NBF, known negative reverse osmosis water. (© I. Finkelde.)

### Commercially Available Test Methods

*Hach*<sup>®</sup> formaldehyde test kit (model FM-1, product no. 2183100).— This method relies on drop count titration and the same sodium sulfite/formaldehyde reaction described above. It utilizes sodium sulfite in solid form, rather than solution. This test expresses results as % formaldehyde.

The test utilizes 1.9 N sulfuric acid ( $\text{H}_2\text{SO}_4$ ) as the titrant, with each drop representing a varying percentage of formaldehyde depending on whether the sample was diluted, either 0.5% or 0.05% formaldehyde per drop. The manufacturer's method was followed, except when measuring percent formaldehyde in ethanol solutions. The solid sodium sulfite in the supplied reagent sachet was insoluble in ethanol, so the contents of the sachet was first dissolved in 10 ml demineralized water and added to the sample before titrating with  $\text{H}_2\text{SO}_4$ . Even though the sample was diluted with the 10 ml solution of water and sodium sulfite,

Table 1. Dilution required to get readable results with formaldehyde test strips.

Formalin (% v/v)	Formaldehyde (% w/v)	Formaldehyde (mg/L)	Dilution factor (RO water)	Formaldehyde after dilution (mg/L)
10.00	4.00	40,000	1:1,000	40
5.00	2.00	20,000	1:500	40
2.00	0.80	8,000	1:200	40
1.00	0.40	4,000	1:100	40
0.50	0.20	2,000	1:100	20
0.20	0.08	800	1:20	40
0.10	0.04	400	1:20	20

the drops were still counted as though each drop represented 0.05% formaldehyde. This adaption of the manufacturer's method should be reliable for concentrations of residual formalin commonly found in ethanol fluid preservatives.

Results obtained in % w/v formaldehyde were converted to % v/v formalin by multiplying by a factor of 2.5, based on the fact that 10% v/v formalin contains 4% w/v formaldehyde gas in water.

*Formaldehyde test strips.*— Two types of commercially available formaldehyde test strips were tested: MQuant<sup>®</sup> formaldehyde test strips (Merck, catalog no. 1100360001) and Quantofix<sup>®</sup> formaldehyde test strips (Macherey-Nagel, ref. 913 28). These test strips give semiquantitative results in mg/L formaldehyde. As the test strips can read only up to 100 mg/L or 200 mg/L formaldehyde, respectively, samples must first be diluted with water to obtain results (Table 1).

After sample dilution with RO water, the manufacturer's instructions were followed. Results are based on comparison of the test strip color with color fields on the container exactly 60 seconds after immersion in the sample. Results as mg/L formaldehyde were converted to % v/v formalin with the following equation, adapted from Kay and Ivison (2007):

$$\% \text{ v/v formalin} = \frac{\text{Result} \times \text{dilution factor} \times 100}{400,000}$$

#### *Testing Each Method with Known Formalin Concentrations*

Known concentrations of unbuffered and neutral buffered formalin (NBF) were made by diluting 37% w/w formaldehyde (Sigma-Aldrich, catalog no. 252549-500ML) with RO water. The 10% v/v NBF was made by diluting this saturated formaldehyde solution 1:9 with RO water and buffering with 4 g/L sodium phosphate monobasic monohydrate (Fisher Chemical, catalog no S369-3) and 6.5 g/L dibasic sodium phosphate anhydrous (Sigma<sup>®</sup> Chemical Company, catalog no S-0876), as described in Simmons (2014).

The known concentrations of NBF, unbuffered formalin, and NBF in 75% v/v ethanol (Pharmco-Aaper, catalog no. 111000200) or 50% v/v isopropanol (Walgreens, UPC 31191720773), shown in Table 2, were tested with each method described above. Higher concentrations of NBF in ethanol and isopropanol were not tested, as these are unlikely to be encountered in alcohol-based fluid preservatives as residual formalin from fixation. The initial ethanol concentration was 75% v/v and the isopropanol was 50% v/v; however, the alcohol concentration was diluted by varying degrees by the addition of the aqueous NBF. Known negatives were also tested with each method. They correspond to RO water, 75% v/v ethanol, and 50% v/v isopropanol.

Table 2. Known concentrations of formalin measured in triplicate with each method.

Known formalin tested (% v/v)	Known formaldehyde (% w/v)	Known formaldehyde (mg/L)	NBF	Unbuffered formalin	NBF in ethanol	NBF in isopropanol
10.00	4.00	40,000	✓	✓	x	x
5.00	2.00	20,000	✓	✓	x	x
2.00	0.80	8,000	✓	✓	✓	✓
1.00	0.40	4,000	✓	✓	✓	✓
0.50	0.20	2,000	✓	✓	✓	✓
0.20	0.08	800	✓	✓	✓	✓
0.10	0.04	400	✓	✓	✓	✓

Tests were conducted with both Quantofix and MQuant formaldehyde test strips on the concentrations of formalin listed in Table 1 without any dilution.

Each method tested yielded results in different units. The sodium sulfite titration method expresses results as % v/v formalin; the Hach formaldehyde test kit expresses results as % w/v formaldehyde; and the Quantofix and MQuant formaldehyde test strips express results as mg/L formaldehyde. For ease of comparison, all the results were converted to % formalin (v/v) and % formaldehyde (w/v) with the equations described above. Three independent tests were conducted on all solutions and an average value calculated.

## RESULTS AND DISCUSSION

The results, as the average of three independent measurements on the known negatives and known concentrations of NBF, unbuffered formalin, NBF in ethanol, and NBF in isopropanol, are presented in Table 3 (sodium sulfite titration method), Table 4 (Hach formaldehyde test kit), and Tables 5 and 6 (Quantofix and MQuant formaldehyde test strips, respectively). Full results are detailed in Supplemental Appendix 2, Tables A2.1, A2.2, A2.3, and A2.4. Table 7 shows results of tests with both brands of formaldehyde test strips on high concentrations of formalin without any dilution.

All methods are sensitive to the effects of other aldehydes such as glutaraldehyde, but given the limited use of such higher aldehydes in collections, this is not considered problematic.

Each of the methods above has advantages and disadvantages that need to be considered when selecting which method to use. These include precision, accuracy, ease of use, sample size, cost, and time. Table 8 details the average time and cost of each test method. All methods are based on detecting color shifts, so adequate color vision is required.

The resolution-related uncertainty of each method is presented in Figures 4, 5, 6, and 7. The bars in Figures 4, 5, 6, and 7 indicate the resolution of each method. Although test strip results align well with nominal concentrations and titration measurements, this is largely an artifact of having prepared known formalin concentration test solutions that corresponded to the measurement steps indicated on manufacturer's test strip color comparison charts. Without this correlation the resolution-related uncertainties in test strip results would be more apparent.

The sodium sulfite titration method has the best resolution of the methods at  $\pm 0.10\%$  v/v formalin and requires a small sample size of 0.75 ml. An additional benefit of this method is that results for titratable acidity are also given. While there is a costly initial set up of equipment, it was overall one of the more cost-effective methods per test for expendable



Table 3. Results of average of three independent measurements on known formalin concentrations with sodium sulfite titration method.

Known concentrations tested	Titrateable acidity <sup>1</sup>	Titration result <sup>2</sup>	Known formalin (% v/v)	Formalin result (% v/v)	Known formaldehyde (% w/v)	Formaldehyde result <sup>3</sup> (% w/v)
<b>Known negatives</b>						
Reverse osmosis water	0	0	0.00	0.00	0.00	0.00
75% v/v ethanol	0	0	0.00	0.00	0.00	0.00
50% v/v isopropanol	1	0.33	0.00	0.03	0.00	0.01
<b>Known formalin concentrations</b>						
<b>NBF (% v/v)</b>						
10.00	10.67	99.67	10.00	9.97	4.00	3.99
5.00	5.67	49.33	5.00	4.93	2.00	1.97
2.00	2	19.67	2.00	1.97	0.80	0.79
1.00	1.67	9.67	1.00	0.97	0.40	0.39
0.50	1	4	0.50	0.40	0.20	0.16
0.20	1	1.33	0.20	0.13	0.08	0.05
0.10	0	1 <sup>4</sup>	0.10	0.10	0.04	0.04
<b>Unbuffered formalin (% v/v)</b>						
10.00	1	100	10.00	10.00	4.00	4.00
5.00	1	50	5.00	5.00	2.00	2.00
2.00	1	20	2.00	2.00	0.80	0.80
1.00	0.33	10.67	1.00	1.07	0.40	0.43
0.50	0	5	0.50	0.50	0.20	0.20
0.20	0	1.33	0.20	0.13	0.08	0.05
0.10	0	1 <sup>4</sup>	0.10	0.10	0.04	0.04
<b>NBF in ethanol (% v/v)</b>						
2.00	2.33	21	2.00	2.10	0.80	0.84
1.00	1	10.33	1.00	1.03	0.40	0.41
0.50	1	5.33	0.50	0.53	0.20	0.21
0.20	0	2.33	0.20	0.23	0.08	0.09
0.10	0	1 <sup>4</sup>	0.10	0.10	0.04	0.04
<b>NBF in isopropanol (% v/v)</b>						
2.00	2.67	20	2.00	2.00	0.80	0.80
1.00	1.33	10.67	1.00	1.07	0.40	0.43
0.50	1	4.33	0.50	0.43	0.20	0.17
0.20	1.33	1.67	0.20	0.17	0.08	0.07
0.10	1	1.33 <sup>4</sup>	0.10	0.13	0.04	0.05

<sup>1</sup> Titrateable acidity = digits of 1.6N NaOH for light blue color shift.

<sup>2</sup> Titration result = digits of 8.0N HCl for light blue color shift.

<sup>3</sup> Calculated from % v/v formalin result.

<sup>4</sup> In some of these tests one-digit 8.0N HCl titrated the sample to clear tone rather than light blue tone. For more accurate results, these samples could have been back titrated with 1.6N NaOH to the light blue color shift.

materials, and this method would be well suited to large-scale projects. Some of the disadvantages of this method are that it takes time to set up equipment and prepare solutions, and there are challenges in maintaining the light blue tone of the pH-adjusted water and sodium sulfite solutions, possibly due to the electrochemical behavior of thymolphthalein within the solution (Uçar and Menek 2003) or from acidification due to carbon dioxide absorption.

The Hach formaldehyde test kit was overall the simplest method to use. All required materials are provided with purchase of the kit. The method recommended by the manu-

Table 4. Results of average of three independent measurements on known formalin concentrations with Hach formaldehyde test kit.

Known concentrations tested	Titration result <sup>1</sup>	Formaldehyde per drop (% w/v)	Known formalin (% v/v)	Formalin result <sup>2</sup> (% v/v)	Known formaldehyde (% w/v)	Formaldehyde result (% w/v)
<b>Known negatives</b>						
Reverse osmosis water	1	0.05	0.00	0.13	0.00	0.05
75% v/v ethanol	1	0.05	0.00	0.13	0.00	0.05
50% v/v isopropanol	0	0.05	0.00	0.00	0.00	0.00
<b>Known formalin concentrations</b>						
<b>NBF (% v/v)</b>						
10.00	7.33	0.50	10.00	9.17	4.00	3.67
5.00	4	0.50	5.00	5.00	2.00	2.00
2.00	14.67	0.05	2.00	1.83	0.80	0.73
1.00	8	0.05	1.00	1.00	0.40	0.40
0.50	4	0.05	0.50	0.50	0.20	0.20
0.20	2	0.05	0.20	0.25	0.08	0.10
0.10	1	0.05	0.10	0.13	0.04	0.05
<b>Unbuffered formalin (% v/v)</b>						
10.00	8.33	0.50	10.00	10.42	4.00	4.17
5.00	5	0.50	5.00	6.25	2.00	2.50
2.00	15.67	0.05	2.00	1.96	0.80	0.78
1.00	9	0.05	1.00	1.13	0.40	0.45
0.50	4	0.05	0.50	0.50	0.20	0.20
0.20	2	0.05	0.20	0.25	0.08	0.10
0.10	1	0.05	0.10	0.13	0.04	0.05
<b>NBF in ethanol (% v/v)</b>						
2.00	15.00	0.05	2.00	1.88	0.80	0.75
1.00	8.33	0.05	1.00	1.04	0.40	0.42
0.50	4.00	0.05	0.50	0.50	0.20	0.20
0.20	2	0.05	0.20	0.25	0.08	0.10
0.10	1	0.05	0.10	0.13	0.04	0.05
<b>NBF in isopropanol (% v/v)</b>						
2.00	13.67	0.05	2.00	1.71	0.80	0.68
1.00	7.33	0.05	1.00	0.92	0.40	0.37
0.50	3.67	0.05	0.50	0.46	0.20	0.18
0.20	2	0.05	0.20	0.25	0.08	0.10
0.10	1	0.05	0.10	0.13	0.04	0.05

<sup>1</sup> Titration result = drops of 1.9N sulfuric acid for clear color shift.

<sup>2</sup> Calculated from % w/v formaldehyde result.

facturer to dissolve the contents of the reagent pillow (solid sodium sulfite) was challenging, and it is recommended to order a lid for the test vial to avoid spills. The sample size required is 1 ml for concentrations within the 0–10% w/v formaldehyde range (0–25% v/v formalin) or 10 ml within the 0–1% w/v formaldehyde range (0–2.5% v/v formalin). The resolution of this method is either  $\pm 0.05\%$  w/v or  $\pm 0.5\%$  w/v formaldehyde ( $\pm 0.125\%$  v/v or  $\pm 1.25\%$  v/v formalin) depending on whether dilutions have been made. This method does not account for any titratable acidity that may be present in the sample, and as results are based on pH shifts, any acidity present in the sample will affect results. This method is not a presence/absence test and was not designed for levels below 0.05% w/v formaldehyde (Hach 2019).

Table 5. Results of average of three independent measurements on known formalin concentrations with Quantofix formaldehyde test strips.

Known concentration tested	Known formaldehyde before dilution (mg/L)	Dilution factor	Known formaldehyde after dilution (mg/L)	Test strip result formaldehyde (mg/L)	Known formalin (% v/v)	Formalin result <sup>1</sup> (% v/v)	Known formaldehyde (% w/v)	Formaldehyde result <sup>2</sup> (% w/v)
Known negatives								
Reverse osmosis water	0	0	0	0	0.00	0.00	0.00	0.00
75% v/v ethanol	0	0	0	0	0.00	0.00	0.00	0.00
50% v/v isopropanol	0	0	0	0	0.00	0.00	0.00	0.00
Known formalin concentrations								
NBF (% v/v)								
10.00	40,000	1:1,000	40	40	10.00	10.00	4.00	4.00
5.00	20,000	1:500	40	40	5.00	5.00	2.00	2.00
2.00	8,000	1:200	40	40	2.00	2.00	0.80	0.80
1.00	4,000	1:100	40	40	1.00	1.00	0.40	0.40
0.50	2,000	1:100	20	20	0.50	0.50	0.20	0.20
0.20	800	1:20	40	40	0.20	0.20	0.08	0.08
0.10	400	1:20	20	20	0.10	0.10	0.04	0.04
Unbuffered formalin (% v/v)								
10.00	40,000	1:1,000	40	40	10.00	10.00	4.00	4.00
5.00	20,000	1:500	40	40	5.00	5.00	2.00	2.00
2.00	8,000	1:200	40	40	2.00	2.00	0.80	0.80
1.00	4,000	1:100	40	40	1.00	1.00	0.40	0.40
0.50	2,000	1:100	20	20	0.50	0.50	0.20	0.20
0.20	800	1:20	40	40	0.20	0.20	0.08	0.08
0.10	400	1:20	20	20	0.10	0.10	0.04	0.04
NBF in ethanol (% v/v)								
2.00	8,000	1:200	40	40	2.00	2.00	0.80	0.80
1.00	4,000	1:100	40	40	1.00	1.00	0.40	0.40
0.50	2,000	1:100	20	20	0.50	0.50	0.20	0.20
0.20	800	1:20	40	40	0.20	0.20	0.08	0.08
0.10	400	1:20	20	20	0.10	0.10	0.04	0.04
NBF in isopropanol (% v/v)								
2.00	8,000	1:200	40	40	2.00	2.00	0.80	0.80
1.00	4,000	1:100	40	40	1.00	1.00	0.40	0.40
0.50	2,000	1:100	20	20	0.50	0.50	0.20	0.20
0.20	800	1:20	40	40	0.20	0.20	0.08	0.08
0.10	400	1:20	20	20	0.10	0.10	0.04	0.04

<sup>1</sup> Calculated from mg/L formaldehyde test strip result.

<sup>2</sup> Calculated from % v/v formalin result.

With both the sodium sulfite titrator method outlined in this paper and the Hach formaldehyde test kit method, there were challenges in interpreting the difference between known negatives and low concentrations of formalin (0.10% v/v). The known negatives were still a light blue color tone, due to the initial pH adjustments of the sodium sulfite solution and pH-adjusted water with the sodium sulfite titrator method, and due to the sodium sulfite and formaldehyde reaction, which caused the solution to become alkaline with the Hach formaldehyde test kit method. With practice, a distinction can be made be-

Table 6. Results of average of three independent measurements on known formalin concentrations with MQuant formaldehyde test strips.

Known concentration tested	Known formaldehyde before dilution (mg/L)	Dilution factor	Known formaldehyde after dilution (mg/L)	Test strip result formaldehyde (mg/L)	Known formalin (% v/v)	Formalin result <sup>1</sup> (% v/v)	Known formaldehyde (% w/v)	Formaldehyde result <sup>2</sup> (% w/v)
Known negatives								
Reverse osmosis water	0	0	0	0	0.00	0.00	0.00	0.00
75% v/v ethanol	0	0	0	0	0.00	0.00	0.00	0.00
50% v/v isopropanol	0	0	0	0	0.00	0.00	0.00	0.00
Known formalin concentrations								
NBF (% v/v)								
10.00	40,000	1:1,000	40	40	10.00	10.00	4.00	4.00
5.00	20,000	1:500	40	40	5.00	5.00	2.00	2.00
2.00	8,000	1:200	40	40	2.00	2.00	0.80	0.80
1.00	4,000	1:100	40	40	1.00	1.00	0.40	0.40
0.50	2,000	1:100	20	20	0.50	0.50	0.20	0.20
0.20	800	1:20	40	40	0.20	0.20	0.08	0.08
0.10	400	1:20	20	20	0.10	0.10	0.04	0.04
Unbuffered formalin (% v/v)								
10.00	40,000	1:1,000	40	40	10.00	10.00	4.00	4.00
5.00	20,000	1:500	40	40	5.00	5.00	2.00	2.00
2.00	8,000	1:200	40	40	2.00	2.00	0.80	0.80
1.00	4,000	1:100	40	40	1.00	1.00	0.40	0.40
0.50	2,000	1:100	20	20	0.50	0.50	0.20	0.20
0.20	800	1:20	40	40	0.20	0.20	0.08	0.08
0.10	400	1:20	20	20	0.10	0.10	0.04	0.04
NBF in ethanol (% v/v)								
2.00	8,000	1:200	40	40	2.00	2.00	0.80	0.80
1.00	4,000	1:100	40	40	1.00	1.00	0.40	0.40
0.50	2,000	1:100	20	20	0.50	0.50	0.20	0.20
0.20	800	1:20	40	40	0.20	0.20	0.08	0.08
0.10	400	1:20	20	20	0.10	0.10	0.04	0.04
NBF in isopropanol (% v/v)								
2.00	8,000	1:200	40	40	2.00	2.00	0.80	0.80
1.00	4,000	1:100	40	40	1.00	1.00	0.40	0.40
0.50	2,000	1:100	20	16.67	0.50	0.42	0.20	0.17
0.20	800	1:20	40	40	0.20	0.20	0.08	0.08
0.10	400	1:20	20	13.33	0.10	0.07	0.04	0.03

<sup>1</sup> Calculated from mg/L formaldehyde test strip result.

<sup>2</sup> Calculated from % v/v formalin result.

tween the color shift of a low-concentration formalin and a known negative, as the low concentration will develop a slightly darker toned blue (see Fig. 3). With these methods, the sodium sulfite titrator method gave more accurate results, as samples were titrated to the light blue color of the color transition point of the thymolphthalein indicator, while the Hach method titrated to a clear tone, which is a more obvious color shift. It is recommended therefore to conduct tests on known negatives and low concentrations before testing fluid preservatives to learn to differentiate between them.

Table 7. Results of testing undiluted concentrations of formalin with Quantofix and MQuant formaldehyde test strips.

Test	Known formaldehyde concentration (mg/L)	Test strip result formaldehyde (mg/L)	Known formalin (% v/v)	Formalin result <sup>1</sup> (% v/v)	Known formaldehyde (% w/v)	Formaldehyde result <sup>2</sup> (% w/v)	Comments
100% formalin solution (Sigma-Aldrich ACS reagent 37% w/w formaldehyde gas in water)							
Quantofix	400,000	~10	100.0	0.0025	40.00	0.001	Color was between 10 mg/L and 0 mg/L result, but not yellow in tone
MQuant	400,000	?	100.0	?	40.00	?	Result was yellow tone, not on comparison chart
10% v/v NBF							
Quantofix	40,000	40	10.00	0.010	4.00	0.004	Color shift to slightly bluer tone and darker splotches on strip; closest to 40 mg/L
MQuant	40,000	100	10.00	0.025	4.00	0.010	Closest to 100 mg/L but had lighter toned pink splotches on strip
5% v/v NBF							
Quantofix	20,000	40	5.00	0.010	2.00	0.004	Closest to 40 mg/L but had some areas of darker splotches
MQuant	20,000	100	5.00	0.025	2.00	0.010	Color was closest to 100 mg/L (dark purple) but had pink tone splotches
2% v/v NBF							
Quantofix	8,000	100	2.00	0.025	0.80	0.010	Closest to 100 mg/L, but more blue tone with splotches
MQuant	8,000	>100	2.00	?	0.80	?	100 mg/L + was much darker in tone than the 100 mg/L comparison area

Table 7. Continued.

Test	Known formaldehyde concentration (mg/L)	Test strip result formaldehyde (mg/L)	Known formalin (% v/v)	Formalin result <sup>1</sup> (% v/v)	Known formaldehyde (% w/v)	Formaldehyde result <sup>2</sup> (% w/v)	Comments
1% v/v NBF							
Quantofix	4,000	200	1.00	0.050	0.40	0.020	Closest to 200 mg/L with splotches
MQuant	4,000	> 100	1.00	?	0.40	?	100 mg/L + was much darker in tone than the 100 mg/L comparison area
0.50% v/v NBF							
Quantofix	2,000	200	0.50	0.050	0.20	0.020	Closest to 200 mg/L with splotches
MQuant	2,000	> 100	0.50	?	0.20	?	100 mg/L + was much darker in tone than the 100 mg/L comparison area
0.20% v/v NBF							
Quantofix	800	> 200	0.20	?	0.08	?	200 mg/L +, was darker in tone than the 200 mg/L comparison area
MQuant	800	> 100	0.20	?	0.08	?	100 mg/L + was much darker in tone than the 100 mg/L comparison area
0.10% v/v NBF							
Quantofix	400	> 200	0.10	?	0.04	?	200 mg/L +, was darker in tone than the 200 mg/L comparison area
MQuant	400	> 100	0.10	?	0.04	?	100 mg/L + was much darker in tone than the 100 mg/L comparison area
RO water (known negative)							
Quantofix	0	0	0.00	0.00	0.00	0.00	Closest match was 0 mg/L on comparison area
MQuant	0	0	0.00	0.00	0.00	0.00	Closest match was 0 mg/L on comparison area

<sup>1</sup> Calculated from mg/L formaldehyde test strip result.

<sup>2</sup> Calculated from % v/v formalin result.

Table 8. Average cost and time per test with each method.

Method	Average time per test (min)	Cost initial equipment setup (US\$)	Cost expendable materials (US\$)	Number of tests	Cost per test <sup>3</sup> (US\$)
Sodium sulfite titration	8	1,427.92	551.10	750	0.73
Hach formaldehyde test kit <sup>1</sup>	6	98.15	50.88 (additional H <sub>2</sub> SO <sub>4</sub> and reagent)	100	0.51–0.98
Quantifix formaldehyde test strips	3–15 (1–5 dilutions may be required to get readable results)	—	79.30 <sup>2</sup>	100	0.79–3.96
MQuant formaldehyde test strips		—	97.30 <sup>2</sup>	100	0.97–4.87

<sup>1</sup> The authors would recommend to also order a lid for the test vial when ordering this kit, to avoid spills when mixing the solid reagent using the manufacturer's published method.

<sup>2</sup> Cost for test strips; if not available at institution, these methods would also incur additional costs for glassware required to make serial dilutions.

<sup>3</sup> All tests also require RO or otherwise purified water.

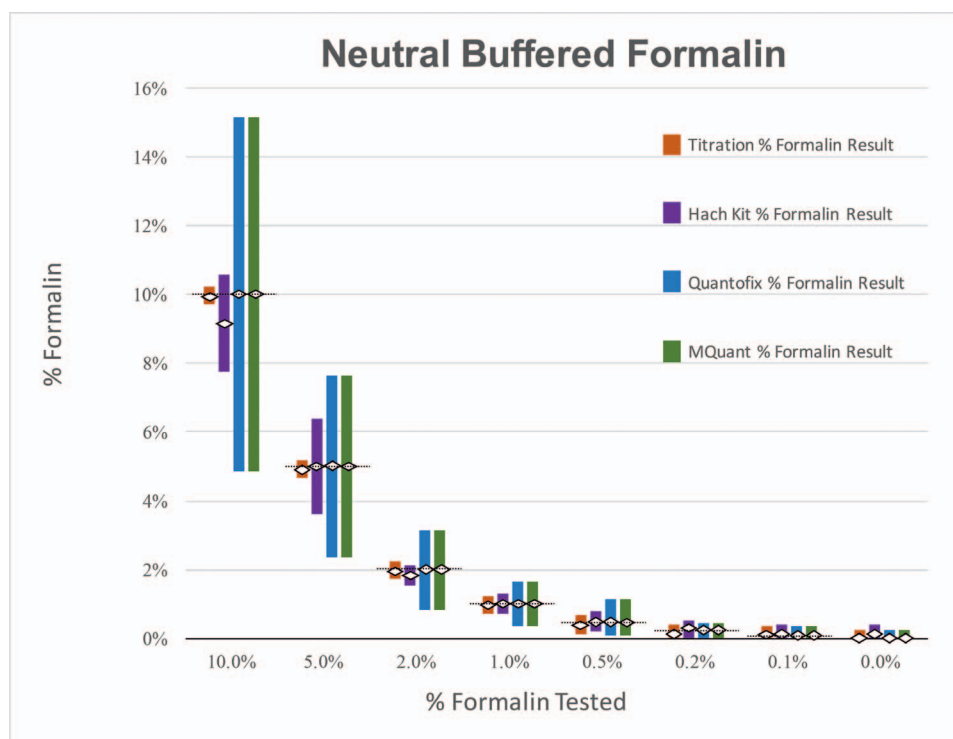


Figure 4. Results of testing known concentrations of NBF (% v/v) with each method. Bars indicate resolution of test methods.

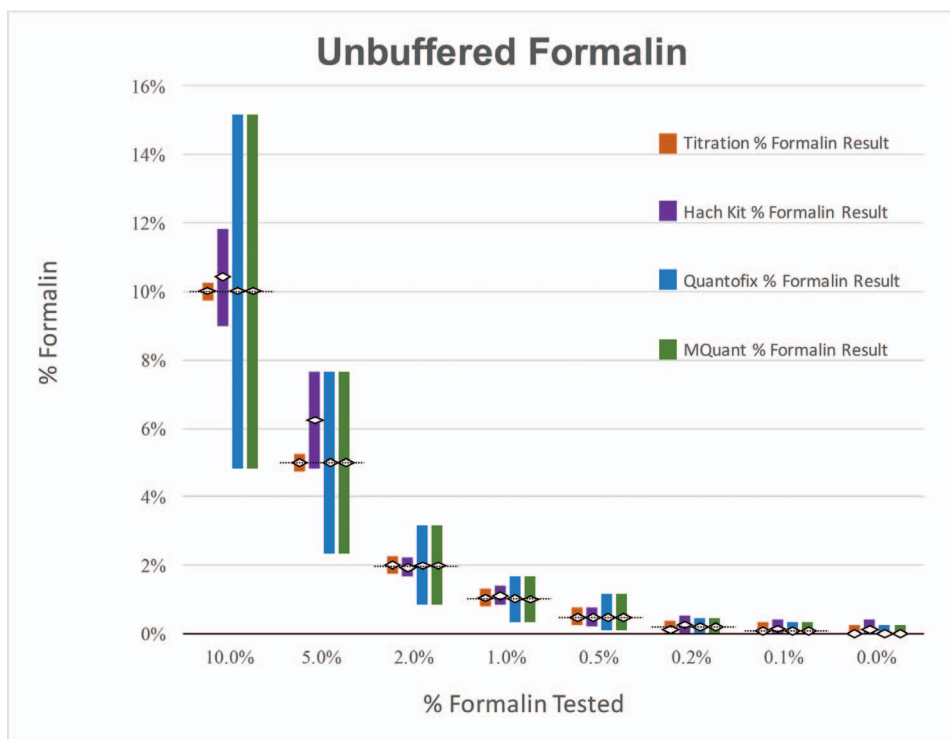


Figure 5. Results of testing known concentrations of unbuffered formalin (% v/v) with each method. Bars indicate resolution of test methods.

While the results presented in Tables 5 and 6 indicate that the formaldehyde tests strips gave the most accurate results, this is due to correspondence of test solution concentrations with available comparison steps and inevitable bias in interpretation of ambiguous results. Both test strips were challenging to use because there is a very limited time period of 1 second to determine the closest color match between the wetted test strip and its color comparison chart. The test strips have the largest gaps in resolution (comparison steps). These vary from  $\pm 0.10\%$  v/v at  $0.10\%$  v/v formalin ( $\pm 100\%$  relative) to  $\pm 5\%$  v/v at  $10\%$  v/v formalin ( $\pm 50\%$  relative), due to the dilutions required and the reporting steps available in the colour comparison charts. Some samples need to be significantly diluted (Table 1) for the test strips to be able to detect the concentration of formalin. Successive serial dilutions may be necessary to get readable results, which can be time consuming and require use of multiple test strips, increasing the cost per sample. The sample size required would range from  $<1$  to 5 ml depending on dilution. The Quantofix formaldehyde test strips can measure a broader range ( $0\text{--}200$  mg/L formaldehyde) compared with the MQuant formaldehyde test strips ( $0\text{--}100$  mg/L formaldehyde). The MQuant formaldehyde test strips need to be stored in a refrigerator.

Both brands of test strips were able to distinguish the known negatives from low concentrations of formalin. There were issues, however, when samples were tested without dilution (Table 7), and this highlights a limitation of the test strips for use in testing fluid preservatives. The test strips gave very inaccurate results for undiluted high concentrations of formalin, with color shifts from the comparison chart and results for low concentrations



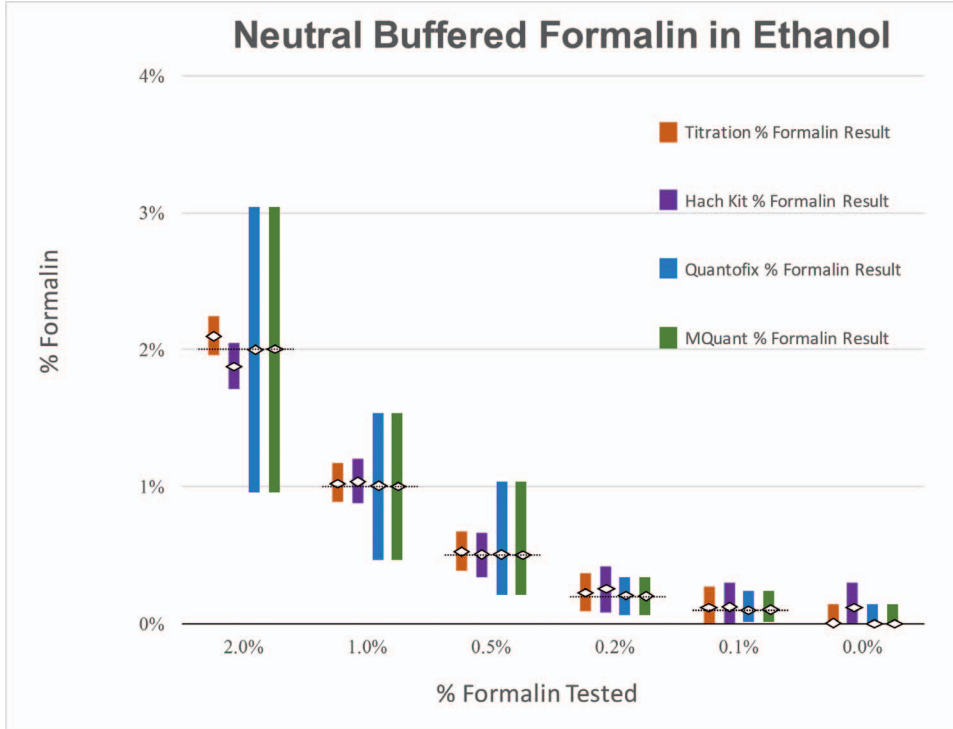


Figure 6. Results of testing known concentrations of NBF in ethanol (% v/v) with each method. Bars indicate resolution of test methods.

when the known concentration was significantly higher. With the Quantofix formaldehyde test strips, results for greater than 200 mg/L formaldehyde were not obtained with formalin concentrations ranging from 100% formalin down to 0.50% formalin. This is an issue as the higher concentrations sometimes gave results on the color comparison scale, which could lead to confusion and misidentification of the formalin concentration.

The most accurate method was the sodium sulfite titrator method. The Hach formaldehyde test kit was the simplest to use, with reasonably accurate results obtained. The issues and challenges presented by both types of formaldehyde test strips, discussed above, highlight the efficacy of the titration-based methods for the quantitative determination of formalin in fluid preservatives. Digital titrators would significantly simplify the process for larger institutions anticipating many tests, while the Hach formaldehyde test kit may suffice for smaller institutions needing fewer tests.

#### FURTHER RESEARCH

It was beyond the scope of this project to test each of these methods on fluid preservatives with specimen-based lipids or other substances, and tests were conducted only on known concentrations and dilutions of formalin. Further research should test the methods described in this paper to determine the effectiveness of each method in determining formalin concentration in actual fluid preservatives in collections. Development of a hybrid of the two titration approaches to optimize the combination of cost, convenience, and accuracy could be explored, as could recently developed paper-based analytical device tests

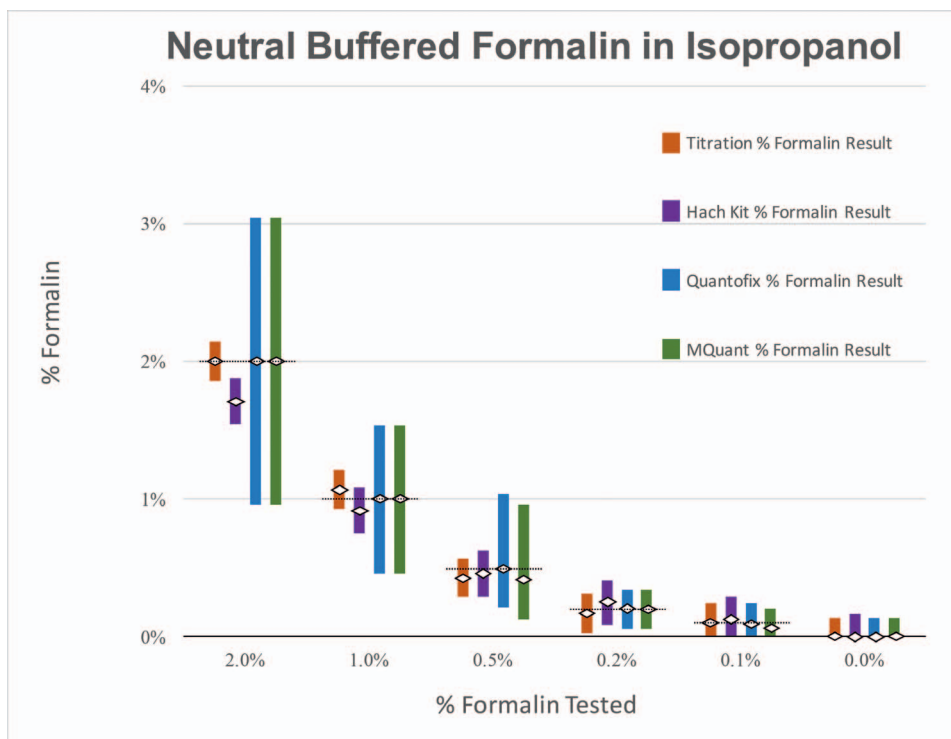


Figure 7. Results of testing known concentrations of NBF in isopropanol (% v/v) with each method. Bars indicate resolution of test methods.

(Taprab and Sameenoi 2019). However, further development of analytical methods might need to take a back seat to assessment of strategically selected collection samples.

## CONCLUSIONS

Formaldehyde is a known carcinogen, so for health and safety reasons or for fluid transfers, it is important to know the concentration of formalin or formaldehyde in solution, be it from preservation in formalin or residual from fixation. The methods presented in this paper assist in determining the concentration of formalin and can be used to better understand the chemistry of fluid preservatives. In conducting rigorous testing with each method on known concentrations of formalin, assessment of advantages and disadvantages of each method were made. These included limits to precision and accuracy due to limited resolution of tests, ease of use, sample size, cost, and time. In considering the advantages and disadvantages of each method, we concluded that titration methods are more effective and easier to use than test strips for determining formalin concentration in fluid preservatives.

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## APPENDIX 1. GUIDELINES FOR THE SODIUM SULFITE TITRATION METHOD

*Initial preparations*

Set the adjustable pipette to deliver 0.75 mL (750  $\mu$ L). If available, a balance accurate to three or more decimal places can be used to verify the setting of the pipette by weighing aliquots of distilled water (0.75 mL aliquots should, on average, weigh 0.748 g).

Install the cartridges in the titrators. Install a straight delivery tube in the NaOH titrant cartridge and a 90° delivery tube in the HCl cartridge. Set aside the caps, and label with NaOH and HCl. Using clamps, support the titrators in positions where they can be easily manipulated and will deliver into a vessel set on the magnetic stirrer. The titrator containing the hydrochloric acid is given the more convenient horizontal position. The equipment set up is shown in Figure 1.

Prepare the pH-adjusted deionized water by adding 10-20 drops of thymolphthalein indicator solution to 250 mL of deionized or RO water. Insert a spin bar in the bottle and place it on the stirrer with the titrator delivery tubes in the solution. This solution needs to be adjusted to the colour transition point of the thymolphthalein indicator. Using the titrator containing NaOH, slowly add NaOH until the solution just turns faint blue. Add one or more HCl titrator digit steps to return the solution to colourless. Very carefully add NaOH, one titrator digit step at a time until the solution again just turns blue. Label the bottle and keep it tightly closed when not in use.

Prepare the 0.5 mol/L sodium sulfite solution by dissolving 15.75 g anhydrous sodium sulfite in 250 mL deionized or RO water. Add 10-20 drops of the thymolphthalein indicator. If the solution is blue in colour, then slowly add HCl until the colour just disappears. Add just enough NaOH to bring back a perceptible light blue colour. Pour into a labelled bottle and keep it tightly closed when not in use. Record the date the solution was prepared on the bottle. Sulfite solutions are oxidized in contact with air. Solutions older than one week should not be used.

The light blue colour of both the pH adjusted water and the sodium sulfite solution will shift back to colourless over time. This is thought to be due to the electrochemical behaviour of thymolphthalein within the solution, as described in Uçar & Menek (2003). Acidification due to carbon dioxide absorption can also contribute to loss of colour. Readjust the colour of the solution every few hours, by adding additional drops of thymolphthalein and readjusting the pH, as required, with NaOH and HCl.

*Analysing a sample*

Conduct all analysis within a fume hood to avoid exposure to hazardous or toxic fumes that may be present in fluid preservatives. Set a clean 25 mL Erlenmeyer flask with a spin bar on the magnetic stirrer. Position both titrator delivery tubes in the flask as near to the bottom as possible and away from the centre area where the spin bar will revolve. Ensure that the readings on both titrators have been zeroed.

Following the manufacturer's directions, use the adjustable pipette to take a 0.75 mL sample of the solution and transfer it to the 25 mL Erlenmeyer flask. Add 3 drops of the thymolphthalein indicator solution. Add 10 mL of the pH-adjusted deionized water. Start the magnetic stirrer. Slowly add NaOH until a just perceptible blue colour appears and remains stable over a five second period, as can be seen in Figures 2A and 2B. Record the reading on the NaOH titrator as the titratable acidity reading then re-zero the titrator.

Titrate acidity complements a pH measurement in characterising the acidity of a solution. It is more meaningful than pH as a measure of past oxidative acidification of solutions and of the capacity of the solution for engaging in further acid-base interactions.

Add 10 mL of the sodium sulfite solution and wait for 30-60 seconds for the reaction to fully develop as a darker blue tone. This is due to the alkalinity of sodium hydroxide formed in the chemical reaction. If a darker blue colour does not appear, then the sample is free of formaldehyde and the formalin concentration can be recorded as 0.0%.

Add HCl from the titrator until the darker blue colour just disappears and the sample is the light blue tone of the colour transition point of the thymolphthalein indicator. This formalin titration process can be seen in Figures 2C and 2D. The darkness of the blue tone that develops when the sodium sulfite is added corresponds to the amount of formalin present in the sample (Figure 3).

Record the titrator reading, divided by ten, as the percent formalin. If you believe that more HCl was added than what was required, then slowly add NaOH to bring back a just perceptible blue colour. Record the percent formalin concentration as the HCl titrator reading divided by ten, minus the NaOH reading divided by fifty. NOTE: If the sample being tested is very yellow in colour, then the colour transition marking the end of the titration will be green to yellow rather than blue to colourless.

The formaldehyde in solution has been converted to a sulfite-formaldehyde addition compound. Refer to local country or state regulations to dispose of waste solutions appropriately, whether that means flushing with dilution or disposal as hazardous waste.

When undertaking the analysis, to get accurate, repeatable and reliable results, ensure no excess NaOH or HCl is on the outside of the titrator delivery tubes. Rinse the delivery tubes into separate small beakers with a wash bottle containing deionized water and tamp dry with tissue. Turn the titrators to expel approximately 5 digits into the tissue and tamp dry before each analysis is undertaken. Re-zero the titrators. Thoroughly rinse the flask. The next sample can now be processed.

To prepare the apparatus for overnight storage, follow the manufacturer's instructions to remove the delivery tubes and store them in separate bottles containing deionized water. Recap the cartridges with the cap supplied. The next day the colour of both the pH adjusted water and the sodium sulfite solutions should also be checked. If they have lost all colour, then additional thymolphthalein indicator should be added, and NaOH should be gradually added to just bring back the just perceptible blue colour of the colour transition point.