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**Objective:** to investigate the effect of the small amount of formaldehyde in the SurePath liquid (< 0.2 %) when establishing a routine protocol for immunocytochemistry.

**Material and methods:** Rest material from unfixed effusions containing either carcinoma cells or a fair number of mesothelial cells (table 1). The cells were transferred to Surepath and left in the liquid for 1, 5 and 12 days before immunostaining. Day 0 equaled a staining protocol not using Surepath for fixation. CK7 was used as a test marker because it was easy to find suitable rest effusion material. Immunostaining was done at several time points and with varying protocols for pretreatment and incubation of CK7 AB. Adding or omitting neutral buffered formalin (NBF) was also recorded (tables 2 and 3). The cytoplasmic staining intensity of carcinoma cells and/or mesothelial cells was evaluated.

Table 1 specimen type

Effusion Nr.	Effusion type	Origin of effusion	Days in refrigerator before transfer to SurePath liquid
M1	Malignant	Ascitic fluid with cells from adenocarcinoma (most probably ovarian origin)	7 days
B1	Benign	Pleural effusion, no clinical information	10 days
B2	Benign	Pericardial effusion, unknown cause	5 days
B3	Benign	Pleural effusion from patient with cardiac failure	1 day

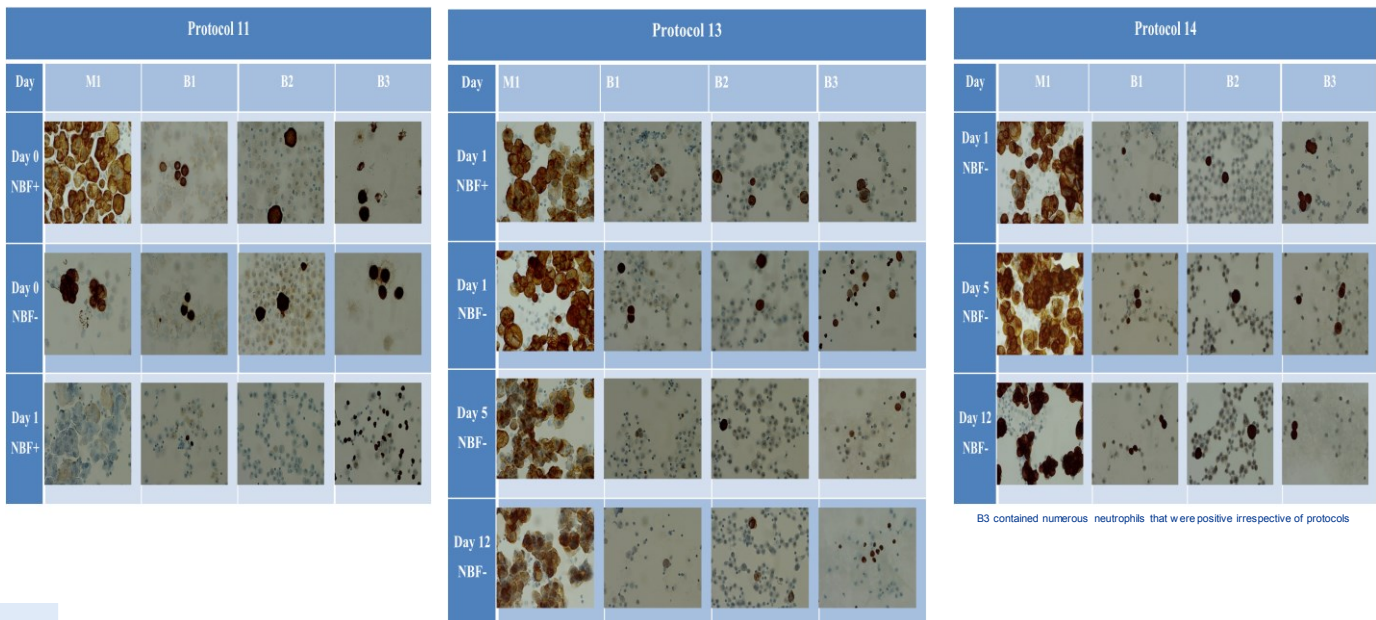
Table 2 protocol specifications

Protocol type	Pretreatment CCl	Incubation anti CK-7
Protocol 11	none	16 min
Protocol 13	8 min	16 min
Protocol 14	30 min	16 min

Table 3 protocols used at specified days

Day 0	Day 1	Day 5	Day 12
Protocol 11, NBF+	Protocol 11 NBF+	Protocol 13 NBF-	Protocol 13 NBF-
Protocol 11, NBF-	Protocol 13 NBF+	Protocol 14 NBF-	Protocol 14 NBF-
	Protocol 13 NBF-		
	Protocol 14 NBF-		

**Results:** details of the staining results for the three protocols are shown below



**Conclusions:** the small amount of formaldehyde in SurePath influences the immunoreactivity of cells in the liquid. Reduced staining intensity may be seen within 5 days of storage in the SurePath liquid. HRT is essential and the pretreatment must reflect the routine storage time of cells in Surepath liquid before eventual immunostaining. Postfixation with NBF should be omitted.