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Clinical Biochemistry xx (2004) xxx–xxx

CLINICAL
BIOCHEMISTRY

Easy fix for clinical laboratories for the false-positive defect with the Abbott AxSym total β -hCG test

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Received 9 September 2003; received in revised form 13 February 2004; accepted 3 March 2004

Abstract

Background: False-positive hCG results can lead to erroneous diagnoses and needless chemotherapy and surgery. In the last 2 years, eight publications described cases involving false-positive hCG tests; all eight involved the AxSym test. We investigated the source of this abundance of cases and a simple fix that may be used by clinical laboratories.

Methods: False-positive hCG was primarily identified by absence of hCG in urine and varying or negative hCG results in alternative tests.

Seventeen false-positive serum samples in the AxSym test were evaluated undiluted and at twofold dilution with diluent containing excess goat serum or immunoglobulin.

Results: We identified 58 patients with false-positive hCG, 47 of 58 due to the Abbott AxSym total hCG β test (81%). Sixteen of 17 of these “false-positive” results (mean 100 mIU/ml) became undetectable when tested again after twofold dilution.

Conclusions: A simple twofold dilution with this diluent containing excess goat serum or immunoglobulin completely protected 16 of 17 samples from patients having false-positive results. It is recommended that laboratories using this test use twofold dilution as a minimum to prevent false-positive results.

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Keywords: hCG; Human chorionic gonadotropin; False-positive; Abbott AxSym; Interference; Heterophilic antibodies

Introduction

hCG is produced in pregnancy, in gestational trophoblastic diseases (GTD) and gestational trophoblastic neoplasms (GTN), and in men with testicular germ cell malignancies. hCG is a perfect tumor marker for GTD and GTN with 100% sensitivity and specificity [1–6]. GTN can be extremely invasive but is very responsive to chemotherapy. A GTN can be dispersed and therefore may not necessarily be detected by imaging methods. Thus, standard practice is to treat women with chemotherapy solely based on persistent elevated hCG results. When this therapy fails to suppress hCG, the hCG result may be

questioned. False-positive hCG results have led to needless therapy [7–17], not just for GTN [7–11,17], but for recurrent testicular malignancy [12], for persistent hydatidiform mole [13], and for ectopic pregnancy [15]. They have also led to the erroneous identification of pregnancy [16].

Multiple reports now show that false-positive hCG tests are due to human heterophilic antibodies interfering with the multiple animal antibody mechanisms of hCG tests and of other assays [14,16,18–21]. This is a particular problem because hCG is one of very few antigens that is either wholly present (as in pregnancy and GTD) or wholly absent (no pregnancy or neoplastic disease).

We found 11 reports of false-positive hCG cases (Medline 1996–2003 and Google 2003) published in 2001–2003, all of which involved false-positive results and needless therapy due to the Abbott AxSym total hCG β test [1,8–17]. According to the CAP, this test is the most commonly used technology and is instituted in 28% of laboratories participating in their survey [21,22]. We investigate here the source for the apparent disproportionate incidence of false-positive

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t1.1 Table 1

t1.2 Tests used by physicians to monitor 58 patients with false-positive hCG results, referred to the USA hCG Reference Service, 1998–2003

Medical treatment incurred because of false-positive hCG results	False-positive concentration at time or closest time to referral to USA hCG Reference Service
(a) Abbott AxSym total β hCG test, used by 28% of laboratories [21,22]	47 cases (81% of total), mean 111 IU/l
t1.4 D&C, Lap, Mtx, AcD, HYS, EMACO, THO	220 IU/l
t1.5 D&C, Lap, Mtx, HYS, EMACO, Coma	68 IU/l
t1.6 D&C, Lap, Mtx, Mtx, AcD, HYS	142 IU/l
t1.7 D&C, Lap, Mtx, HYS	17 IU/l
t1.8 D&C, Lap, HYS, BSO	150 IU/l
t1.9 D&C, Lap, Mtx, BSO	145 IU/l
t1.10 D&C, Lap, Mtx, HYS, BSO	81 IU/l
t1.11 D&C, Lap, Mtx, AcD, EMACO	558 IU/l
t1.12 D&C, Lap, Mtx, EMACO	80 IU/l
t1.13 D&C, Lap, Mtx, AcD	22 IU/l
t1.14 D&C, Lap, Mtx, AcD	21 IU/l
t1.15 D&C, Lap, Mtx, AcD	110 IU/l
t1.16 D&C, Lap, Mtx, AcD	114 IU/l
t1.17 History of hydatidiform mole, Mtx, AcD	115 IU/l
t1.18 D&C, Mtx, D&C, Lap, Mtx	20 IU/l
t1.19 D&C, Lap, Mtx, Mtx	97 IU/l
t1.20 D&C, D&C, Lap, Mtx	122 IU/l
t1.21 D&C, Mtx, AcD	14 IU/l
t1.22 D&C, Lap, Mtx	60 IU/l
t1.23 D&C, Lap, Mtx	139 IU/l
t1.24 D&C, Lap, Mtx	402 IU/l
t1.25 D&C, Lap, Mtx	37 IU/l
t1.26 D&C, Lap, Mtx	607 IU/l
t1.27 D&C, Lap, Mtx	300 IU/l
t1.28 D&C, Lap, Mtx	21 IU/l
t1.29 D&C, Mtx	202 IU/l
t1.30 D&C, Mtx	18 IU/l
t1.31 D&C, Mtx	81 IU/l
t1.32 D&C, Mtx	170 IU/l
t1.33 D&C, Mtx	124 IU/l
t1.34 D&C, Mtx	70 IU/l
t1.35 D&C, Mtx	21 IU/l
t1.36 History of hydatidiform mole, Mtx	17 IU/l
t1.37 History of hydatidiform mole, Mtx	8 IU/l
t1.38 D&C, Lap	14 IU/l
t1.39 D&C, Lap	23 IU/l
t1.40 D&C, Lap	24 IU/l
t1.41 D&C	32 IU/l
t1.42 D&C	93 IU/l
t1.43 D&C	143 IU/l
t1.44 GTN considered, no therapy	53 IU/l
t1.45 GTN considered, no therapy	174 IU/l
t1.46 GTN considered, no therapy	25 IU/l
t1.47 GTN considered, no therapy	224 IU/l
t1.48 GTN considered, no therapy	20 IU/l
t1.49 Male patient, recurrence of TGCM considered, no therapy	15 IU/l
t1.50 Male patient, TGCM considered, no therapy	38 IU/l
(b) Dade Dimension RXT intact hCG test, used by 21% of laboratories [21,22]	2 cases (3.4% of total), mean 53 IU/l
t1.52 D&C, Mtx	84 IU/l
t1.53 D&C, Mtx	23 IU/l

Table 1 (continued)

Medical treatment incurred because of false-positive hCG results	False-positive concentration at time or closest time to referral to USA hCG Reference Service	
(c) Beckman Access-2 total β hCG test, used by 15% of laboratories [21,22]	0 cases	t1.55
(d) Bayer ADVIA Centaur total hCG test, used by 7.5% of laboratories [21,22]	3 cases (5.2% of total), mean 19 IU/l	t1.56
D&C, Mtx	20 IU/l	t1.57
GTN considered, no therapy	18 IU/l	t1.58
GTN considered, no therapy	18 IU/l	t1.59
(e) Ortho Vitros ECi total hCG test, used by 6.1% of laboratories [21,22]	2 cases (3.4% of total), mean 48 IU/l	t1.60
GTN considered, no therapy	55 IU/l	t1.61
History of hydatidiform mole, Mtx, AcD	41 IU/l	t1.62
(f) DPC Immulite/Immulate 2000 hCG test, used by 4.1% of laboratories [21,22]	0 cases	t1.63
(g) Roche Elecsys E170 intact hCG test, used by 3.6% of laboratories [21,22]	0 cases	t1.64
(h) Bayer ACS180 total hCG test, used by 3.3% of laboratories [21,22]	2 cases (3.4% of total), mean 39 IU/l	t1.65
History of hydatidiform mole, no therapy	65 IU/l	t1.66
Mtx, AcD	20 IU/l	t1.67
(i) All other assays (one case with Tosoh Nexia, one with Bayer Immuno-1)	2 cases (3.4% of total), mean 30 IU/l	t1.68
D&C, Lap	53 IU/l (Immuno 1)	t1.69
GTN considered, no therapy	7 IU/l (Nexia)	t1.70

The table shows test used and false-positive results reported to physician at or closest to the time of referral to the USA hCG Reference Service. The table also summarizes the medical treatment incurred by the patient because of the continuous false-positive hCG test result. D&C is dilation and curettage; Lap is laparoscopy; Mtx is methotrexate chemotherapy; AcD is actinomycin D chemotherapy; EMACO is five agent cytotoxic chemotherapy (Etoposide, Mtx, AcD, alternating with cyclophosphamide and vincristine); HYS is hysterectomy; BSO is bilateral salpingo-oophorectomy; THO is thoracotomy; and Coma is diabetic coma due to destruction of Islet cells by chemotherapy. TGCM is testicular germ cell malignancy and GTN is gestational trophoblastic neoplasm. Data are presented in order of proportion of laboratories using a test where the tests are the first most common, second, third, fourth, fifth, sixth, and seventh most commonly used hCG tests in North America [21,22].

In a *t* test comparing the mean false-positive result in the Abbott AxSym with the mean result in all other tests $P < 0.05$.

results with this assay and describe simple methods for laboratories using the Abbott AxSym total hCG β to identify and avoid false-positive results.

Methods

Serum samples were accumulated as part of the ongoing practice of the USA hCG Reference Service. After obtain-

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ing approval from the University of New Mexico Health Sciences Center Human Research Review Committee (HRRC #02-548), we disclose USA hCG Reference Service Results and hCG results reported at each referring center.

The presence of false-positive hCG was identified by the USA hCG Reference Service as described previously [1,7]. Briefly, false-positive results were demonstrated by at least three of four observations: (a) presence of hCG or related molecule immunoreactivity in serum but not urine; (b) results varying more than fivefold in multiple different hCG assays or immunoreactivity not detected in hCG Reference Service assays, the DPC Immulite hCG and free β assay (DPC, Los Angeles CA), or the "in-house" microtiter plate 2 monoclonal antibody intact hCG and β -core fragment assays, as described previously [1,7]; (c) hCG immunoreactivity suppressed by heterophilic antibody blocking agent HBT (Scantibodies Inc., San Diego, CA) using either the DPC Immulite hCG or free β assay or the "in-house" microtiter plate 2 monoclonal antibody intact hCG assay; and (d) the finding of elevated urine β -core fragment in serum but not urine using the "in-house" microtiter plate 2 monoclonal antibody assay as described previously [1,7].

In six cases of false-positive hCG, serum samples were also analyzed on the Abbott AxSym total hCG β test at the referring center, both undiluted and twofold diluted with Abbott AxSym diluent. This includes the two cases identified by Giannopoulos et al. [16]. One case was run on the AxSym, undiluted and at twofold dilution at the initiative of the laboratory. A further 10 serum samples, demonstrated as false-positive by the four criterion described in the previous paragraph in the Abbott AxSym total hCG β test (Table 2), were tested undiluted and at twofold dilution.

Data on the numbers of laboratories using a specific professional laboratory hCG test were obtained from the combination of three College of American Pathologists surveys, the Excel survey 2002 and 2003 [21], and the K/KN-A Ligand (General) survey [22]. Data are the most recent available, from the C-15 [21] and K-05 [22] surveys involving 4554 testing laboratories.

In all cases, the specific hCG test used by the referring physician to test the patient, and the most recent false-positive result, was confirmed with the referring center's laboratory. All statistics were determined in the Microsoft Excel spreadsheet, including means and Student's *t* test.

111 Results

112 Table 1 summarizes the 5-year experience of the USA
113 hCG Reference Service with cases with false-positive hCG
114 results. It also summarizes medical treatment resulting
115 solely from the false finding of elevated hCG results. As
116 shown, in order of test use according to CAP surveys

[21,22], 47 cases with false-positive results (81%) were
from centers using the Abbott AxSym total hCG β test; 2
false-positive cases (3.4%) involved the Dade Dimension
RXT intact hCG test; no false-positive results were recorded
with the Beckman Access-2 total β hCG test; 3 false-positive
cases (5.2%) involved the Bayer ADVIA Centaur total hCG
test; 2 false-positive cases (3.4%) involved the Ortho Vitros
Eci assay; no false-positive results were recorded with the
DPC Immulite/Immolute 2000 or the Roche Elecsys E170
Intact hCG test; and 2 false-positive cases (3.4%) involved
the Bayer ACS180 total hCG test. The remaining two false-
positive cases came from centers using the Tosoh Nexia and
Bayer Immuno-1 total hCG tests. No false-positive results
were recorded with Abbott's new hCG test, the Abbott
Architect hCG test, a newly designed immunometric assay,
or with any other manufacturer's assay. It should be noted,
however, that while the Abbott AxSym and most of the
other tests have been available for the 5 years duration of
this study, one of assays, the Roche Elecsys E170, has been
in use for less than 5 years. It is not possible, however, to
project the Roche result since one cannot amplify zero (0
false-positives).

We considered the relationship between the proportion of
false-positive results due to a specific test and the proportion
of laboratories using a specific test: Abbott AxSym total
hCG β test (ratio is 81% \div 28% or 2.9*X*, where *X* is the
multiplicand of the share of the market); Bayer ACS180
total hCG test (ratio 0.94*X*); Bayer ADVIA Centaur total
hCG test (ratio 0.69*X*); Ortho Vitros Eci total hCG test
(0.56*X*); Dade Dimension RXT intact hCG (0.16*X*); Beck-
man Access-2 total β hCG test (0*X*); DPC Immulite hCG
(0*X*); and Roche Elecsys E170 intact hCG (0*X*). The Abbott
AxSym total hCG β test accounts for a high number of false-
positive hCG cases, clearly disproportionate to its share of
the market. In addition, the average false-positive result in
the Abbott AxSym total hCG β test (mean 111 mIU/ml) is
significantly higher than of all other test false-positive
results combined (*t* test, *P* < 0.05). Of the 47 cases with
false-positive results in the Abbott AxSym total hCG β test,
40 (85%) needlessly received surgery or chemotherapy. Of
the 11 other false-positive test cases, only six (55%)
received needless therapy.

We investigated the cause and potential remedies for
false-positive results in the Abbott AxSym total hCG β test.
Seventeen serum samples from individuals with false-pos-
itive hCG results were tested in the Abbott AxSym total
hCG β test with no dilution and after twofold dilution with
the supplied Abbott AxSym diluent (at both referring center
laboratories, external laboratories under instruction of the
USA hCG Reference Service, and as ordered by the USA
hCG Reference Service). As shown in Table 2, 16 of the 17
serum samples, clearly positive for hCG when tested with
no dilution (mean concentration 100 IU/l), became unde-
tectable (sensitivity of Abbott AxSym total hCG β test is 2
IU/l) after a twofold dilution. The remaining sample gave a
result of 8.5 IU/l (versus 34 IU/l) when twofold diluted,

t2.1 Table 2

Seventeen serum samples from patients with proven false-positive hCG result in the Abbott AxSym assay, tested undiluted and twofold diluted to illustrate false-positive hCG result

Sample	Abbott AxSym, no dilution (IU/l)	Abbott AxSym twofold dilution (IU/l)
t2.4	1	607
t2.5	2	174
t2.6	3	224
t2.7	4	30
t2.8	5	17
t2.9	6	36
t2.10	7	17
t2.11	8	7
t2.12	9	16
t2.13	10	45
t2.14	11	34
t2.15	12	55
t2.16	13	62
t2.17	14	110
t2.18	15	98
t2.19	16	87
t2.20	17	83
t2.21	Mean concentration	100

Sample 1 is the data reported to the USA hCG Reference Service. Samples 2–11 were tested at request of the hCG Reference Service (Abbott AxSym results determined by an independent laboratory in Albuquerque). Samples 12–17 are communicated reports (USA hCG Reference Service suggests to external laboratory considering Abbott AxSym false-positive problems to repeat test with twofold dilution, results then communicated back to reference service).

t2.22

173 indicating a multifold reduction of false-positive hCG
174 results.

175 **Discussion**

176 In 1998, we described three false-positive cases, two of
177 the three were from physicians using the Abbott AxSym test
178 [23]. In 1999, we had data on six false-positive cases, five of
179 six were monitored using the AxSym [17]. In 2000, we
180 described 12 false-positive cases, 11 of the 12 false were
181 due to the AxSym [7]. Now, in 2003, we report 58 patients,
182 47 of 58 due to the AxSym (81%). In the majority of cases
183 (85%), patients had needless therapy because of the false-
184 positive hCG values reported by clinical laboratories. Con-
185 sidering the use of this assay in the USA, 28% of labora-
186 tories [21,22], this seemed an extraordinary high incidence.
187 In support of this 5-year observation by the USA hCG
188 Reference Service, all recently published articles (11 of 11
189 article) describing false-positive hCG cases (between 2001
190 and 2003) involved patients tested falsely with the Abbott
191 AxSym total hCG β assay [1,8–17]. All of these findings
192 clearly show that the Abbott AxSym total hCG β test is
193 responsible for a disproportionately large number of false-
194 positive cases. Furthermore, the average false-positive result
195 in the Abbott AxSym total hCG β test was statistically
196 higher than false-positive results in all other tests combined
197 and led to a higher proportion of patients having needless

surgery and chemotherapy than for all other tests combined. 198
The Abbott AxSym total hCG β test clearly causes an 199
unacceptable high number of false-positive hCG tests. 200

The Abbott AxSym total β -hCG test instruction manual 201
recommends no dilution for serum samples containing up to 202
1000 IU/l. This covers a major proportion of hCG determi- 203
nations. The manual describes the three components in the 204
reagent pack: (1) a monoclonal anti- β hCG coated on micro- 205
particles in Tris buffer with protein stabilizers and azide 206
preservative; (2) a purified goat anti- β hCG conjugated to 207
alkaline phosphatase in Tris buffer with protein stabilizers 208
and azide preservative; and (3) a sample diluent containing 209
bovine and goat serum and azide preservative. The animal 210
serum or immunoglobulin, to protect from heterophilic 211
antibody interference, is only described as present in the 212
diluent. As such, undiluted samples would have no animal 213
immunoglobulin protection against false-positive results. 214
All of the false-positive samples described in Table 1 were 215
<1000 IU/l, so were likely tested undiluted or without 216
protection. In one false-positive case, for example, the 217
AxSym test gave a result of 607 IU/l. When this serum 218
sample was tested at twofold dilution, hCG immunoreactiv- 219
ity was undetectable (reported at <5 IU/l). A twofold 220
dilution is the minimal practical dilution. Clearly, the 221
addition of a minimal amount of diluent animal antibodies 222
completely suppressed the false-positive result. As de- 223
scribed, a simple twofold dilution with Abbott AxSym 224
diluent blocked false-positive results completely in 16 of 225
17 cases and partially block results in one case. This 226
confirms that the absence of diluent or nonspecific animal 227
immunoglobulins leads to false-positive hCG results. The 228
lack of protection of undiluted samples against false-posi- 229
tive results, by the addition of nonspecific animal immuno- 230
globulin, is clearly a defect in the design of the Abbott 231
AxSym total β -hCG test. This defect has been independen- 232
tly described by Pesce [13] and by Giannopoulos et al. [16]. 233

Excess animal serum and immunoglobulins or hetero- 234
philic antibodies blocking agents should be a requisite 235
component added to all samples, regardless of dilution, 236
either in the capture or tracer (also called conjugate) 237
antibody components. As published, the DPC Immulite/
238 Immulite 2000 and Roche Elecsys E170 tests use false-
239 positive blocking agents in all antibody mixtures [13].
240 Applicable technical staff informed us that the Dade Di-
241 mension RXT intact hCG assay, Ortho Vitros ECi, Beckman
242 Access-2 total β hCG assay, and Bayer Diagnostics ADVIA
243 Centaur and ACS180 assay all use blocking in either or both
244 the capture and tracer component of the assay to avoid all
245 false-positives at all dilutions. As such, in seven of the eight
246 of the most commonly used hCG assay by laboratories in
247 the United States and Canada, protection is present in a
248 requisite component regardless of sample dilution. This is
249 not the situation with the Abbott AxSym total β -hCG test in
250 which the blocking agent (animal serum) is in the diluent.
251 This leaves undiluted samples virtually unprotected. This is
252 an irregular design, making the assay defective. Considering
253

254 the large number of people being hurt because of this assay
255 defect, receiving needless surgery or chemotherapy (40
256 cases described here, others in the cited papers [8–17]
257 and an unknown number not reported), Abbott should
258 consider its ethical responsibility to the public and fix the
259 assay or withdraw it.

260 Twenty years ago, competitive hCG β radioimmunoas-
261 says were used. Using these assays, which use limiting
262 antibody, lack of parallelism with dilution was an indicator
263 of a cross-reacting antigen such as a heterophilic antibody
264 [24–28]. The excess antibody design of modern immuno-
265 metric assays makes parallelism with dilution less of an
266 indicator. In our experience, most cases of false-positive
267 results decrease parallel to dilution.

268 Giannopoulos et al. [16], concerned by the Abbott
269 AxSym false-positive problem, started at the initiative of
270 the USA hCG Reference Service to run the test both
271 undiluted and at twofold dilution for a period of 14 months.
272 During this time, 2 patients out of 2860 tested were
273 identified by this means (twofold dilution result compared
274 to undiluted result) as having false-positive results with no
275 dilution yet not detected at twofold dilution). False-positive
276 results were confirmed using urine samples and three other
277 serum assays [16]. This indicates that approximately 1 in
278 1430 tests yield false-positive results. Considering that
279 approximately 7 million women achieve pregnancy or each
280 year in the United States and Canada [29], that the Abbott
281 Axsym total β -hCG test is used in 28% of laboratories
282 [21,22], and 1 in 1430 false-positive rate, then approximate-
283 ly 1371 women are likely to get erroneously identified with
284 pregnancy or ectopic pregnancy each year due to the
285 limitation of the Abbott AxSym test. This estimate does
286 not include those incidentally checked for pregnancy before
287 surgery or X-ray, the most common reason for initial hCG
288 tests among the 58 false-positive cases described here. It
289 also does not include the women with history of hydatidi-
290 form mole or other malignancy or men with testicular
291 malignancy having hCG tests and falsely diagnosed with
292 recurrence. As such, the true incidence of Abbott AxSym
293 false-positive cases is likely to be significantly greater.

294 However, when one examines or analyzes these numbers,
295 a large number of people are being erroneously diagnosed
296 and treated because of a clear deficiency with one hCG test.
297 This test can easily be fixed by Abbott Laboratories, by
298 adding serum, nonspecific antibodies or other blocking
299 agents to a requisite component (capture or conjugate anti-
300 body wells) of the test pack. As shown here, and confirmed in
301 an abstract by Giannopoulos et al. [16], an easy fix for clinical
302 laboratories that continue to run the Abbott AxSym total β -
303 hCG test is either (1) running all samples undiluted and at
304 twofold dilution to identify false-positives, or (2) limiting the
305 test to twofold dilution avoiding undiluted samples and most
306 false-positive results. The test currently has a sensitivity of 2
307 IU/l but recommends stating negative pregnancy results at <5
308 IU/l (Abbott Axsym total β -hCG test instruction manual).
309 This detection level (5 mIU/ml) should not be affected by

running the test at twofold dilution. The twofold dilution is
not an automatic dilution programmed in this assays soft-
ware. It needs to be performed manually and will take time.
Laboratories, however, need to also take responsibility in
avoiding the numerous false results that occur. They need to
consider legal responsibilities and the possibility of being
sued for false-positive hCG results.

In 2000, in response to the false-positive hCG test prob-
lem, Abbott Laboratories added warning and limitation
notices to its instruction manual. Warning letters have also
been sent by Abbott to all laboratories. It is noted in these
warnings that this test should only be used for pregnancy and
not for GTN. Most of the cases, however, started off with
multiple false-positive pregnancy test results before pregnan-
cy, and ectopic pregnancy was excluded and GTN was
assumed. This warning will not prevent false-positive hCG
results in GTN cases. In particular, it will not prevent false-
positive pregnancies and assumptions of ectopic pregnancies.

Considering the data described here, limiting the test to
twofold dilution, as the least dilution, will largely surmount
the false-positive defect and greatly decrease the occurrence
of false-positive hCG results. Alternatively, running samples
undiluted and at twofold dilution would identify most false-
positive cases.

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