Supplementary Information for

An Integrated Paper-based Microfluidic Platform for Screening of Early-Stage Alzheimer's Disease by Detecting of Aβ42

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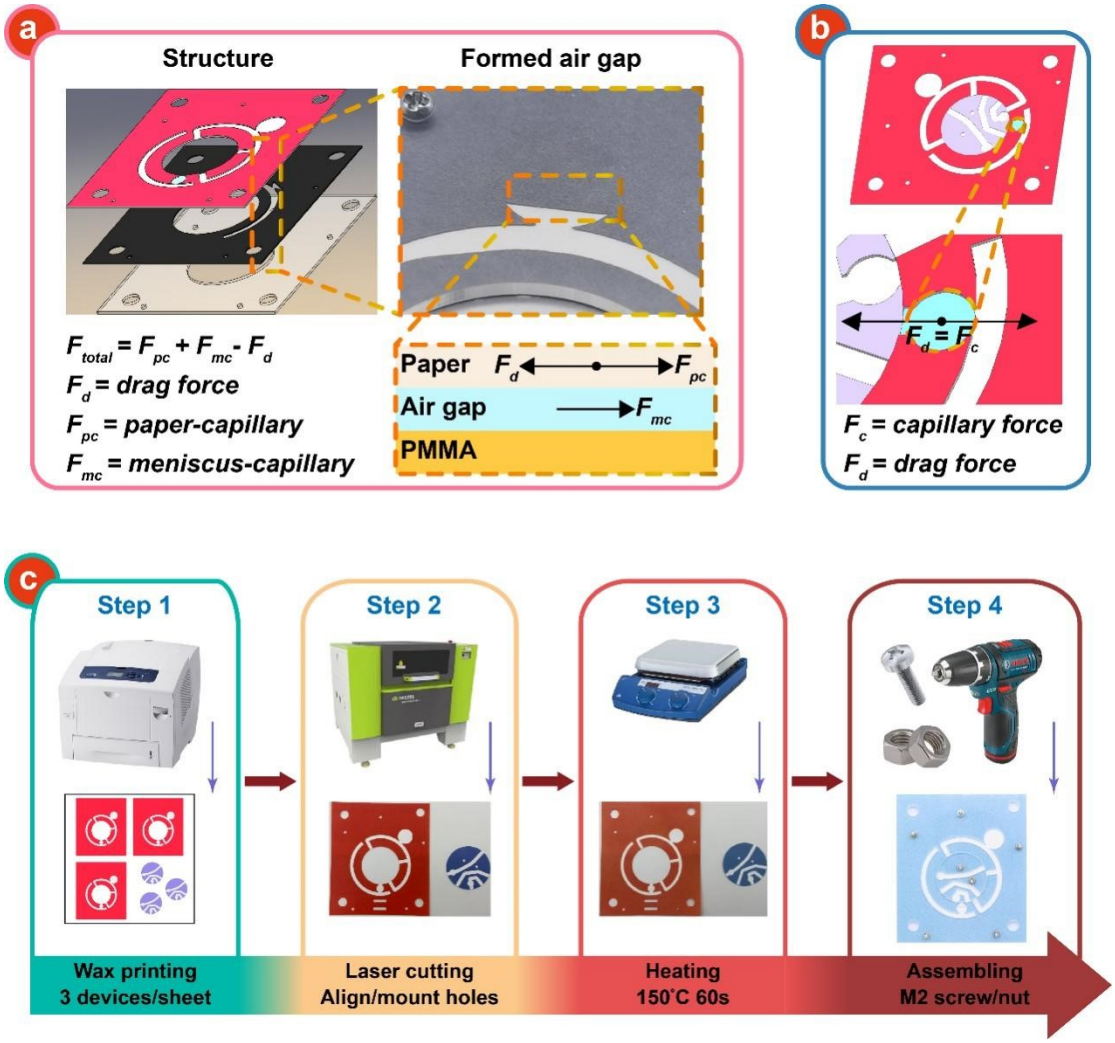

Figure S1. Additional features and mechanisms of the paper-based microfluidic chip device, along with fabrication process. (a) The structure and mechanism for flow speeding up. An additional meniscus-capillary force was introduced by the air gap formed between paper channels and supporting PMMA separated by a carved wax paper. (b), Illustration of stop mechanism of the reagents flushed into the test zone, utilizing the equilibrium between the liquid's viscous drag force and the capillary force of both paper and air gap. (c), The fabrication process of the paper-based device. Step 1: Transfer designed patterns to the paper by wax printing; Step 2: detach patterns and drill align/mount holes through laser cutting; Step 3: heat the paper to form hydrophobic boundaries by melting printed wax throughout paper; Step 4: assemble paper and two layers of PMMA cases with M2 screws and nuts.

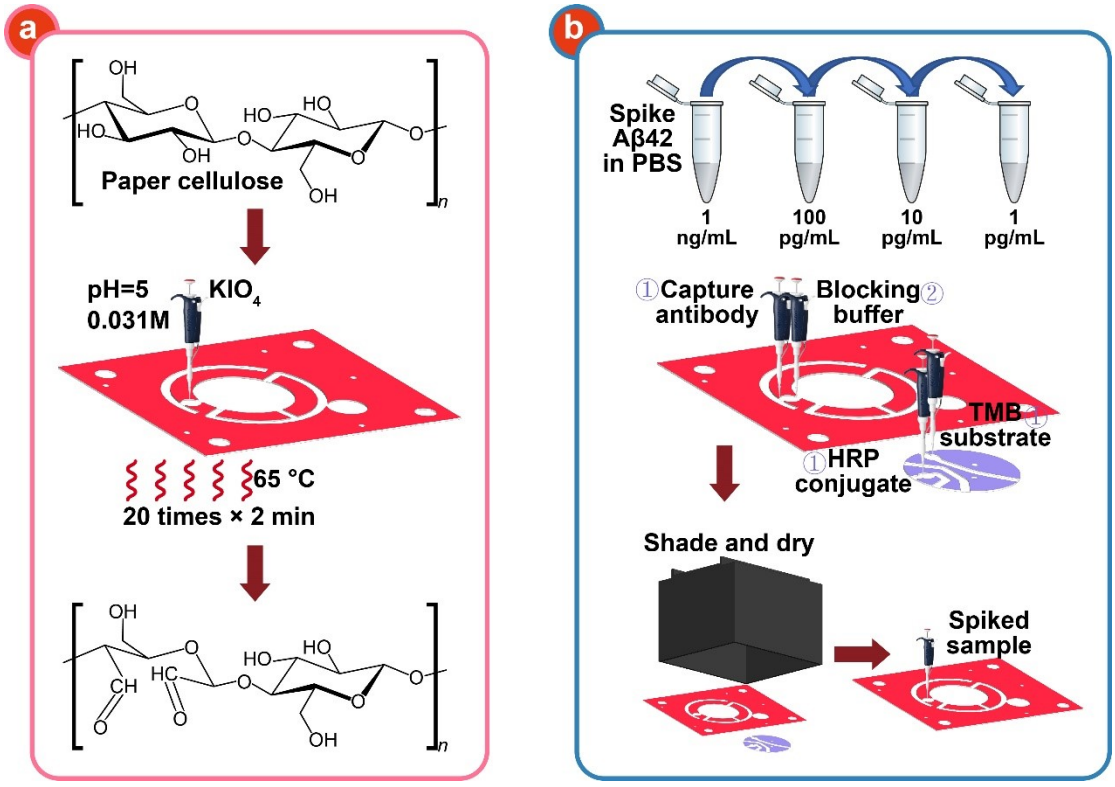

Figure S2. Illustration of preparations for paper-based chips before use. (a) Biofunctionalization of the test zone by potassium periodate (KIO_4) to strengthen binding between proteins and paper: To perform this process, we spotted KIO_4 aqueous solution (0.031M, pH=5) with 1.5 μL under 65 ℃, repeating every 2 min for 20 times. Hydroxyl groups on cellulose paper changed to aldehyde groups, capable to achieve strong binding of proteins via covalent bonds. (b) Preparation of spiked samples for obtaining calibration curve and loading reagents on the paper-based chip: Firstly, capture antibody (3 μL) was added into the test zone, while HRP conjugated antibody (3 μL) and TMB substrate (3 μL) were added into storage 1 and 2 respectively, after which paper-based chip was dried for 10 min under room temperature; Secondly, blocking buffer (3 μL) was added into the test zone and then dried for 10 min under room temperature; Thirdly, spiked sample (3 μL) was added into the test zone and then dried for 10 min under room temperature. The paper-based chip was shaded by a 3D printed apparatus to avoid light decomposition of TMB substrate during all drying steps.

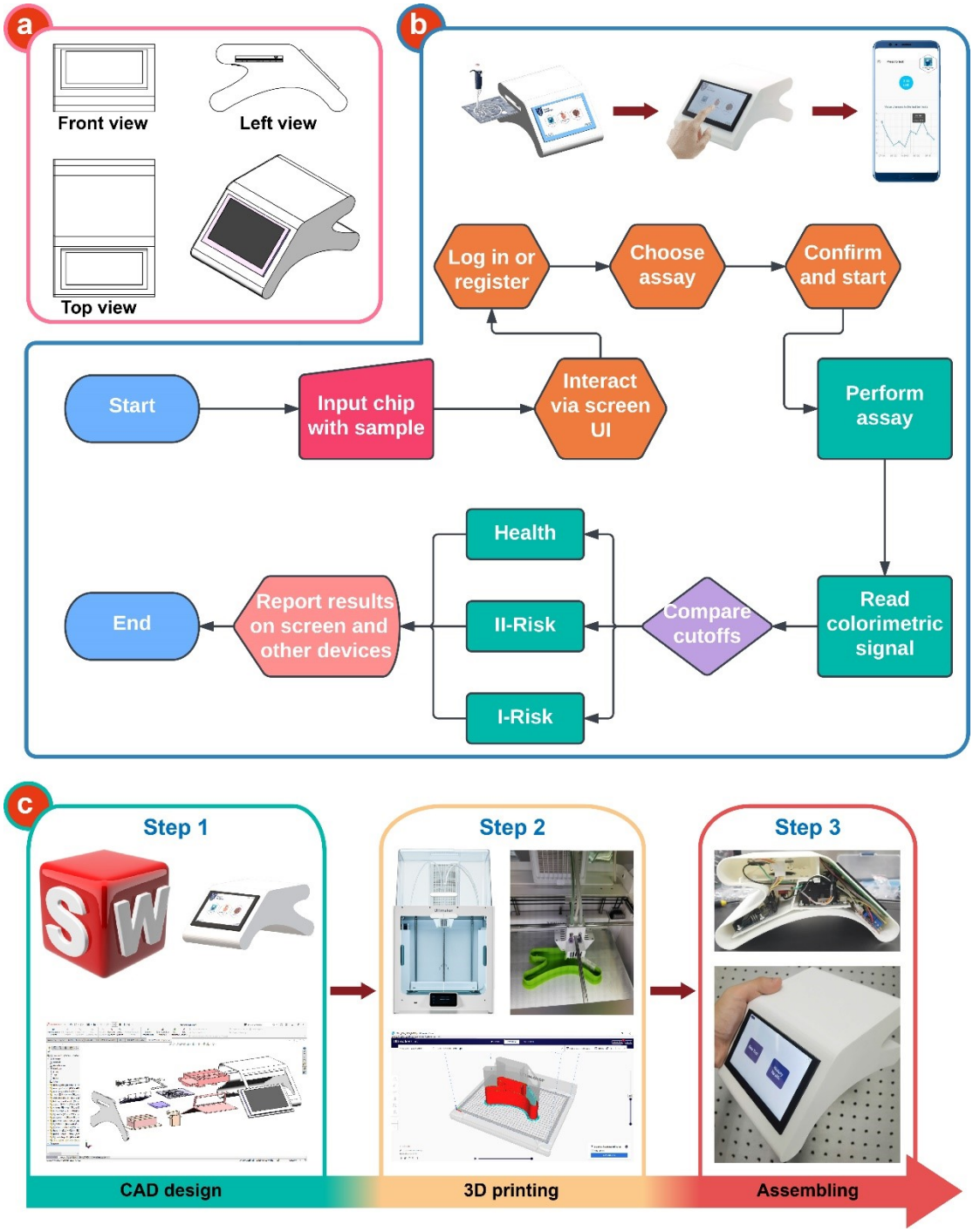

Figure S3. Design of appearance, functions, and interactions of the RAPID platform, along with fabrication and assembling process. (a), Three-view drawing of the appearance of the RAPID platform. (b), Flowchart of interactions between users and the RAPID platform. (c), Fabrication and assembling process of the RAPID platform. Step 1: Computer-aided design (CAD) design for the appearance and structure of the RAPID platform by Solidworks; Step 2: Fabrication of shells and supporting-frame structures of the RAPID platform by 3D printing; Step 3: Assembling of 3D-printing structures with electronic components: microcontroller, screen, sensor, etc.

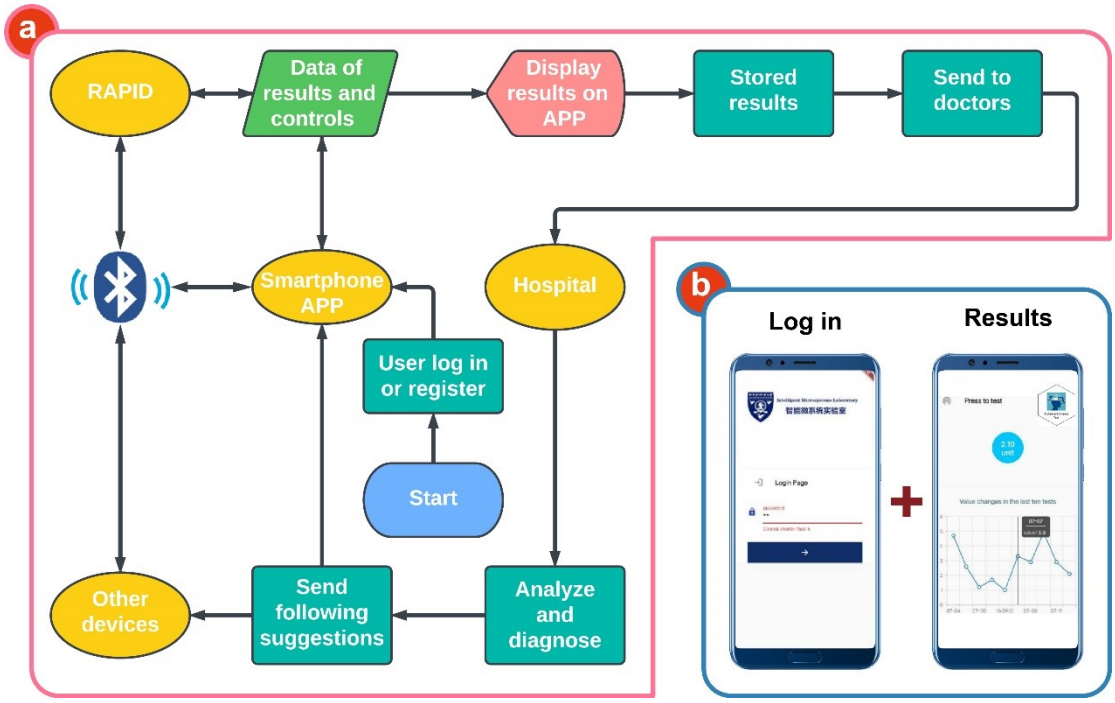

Figure S4. Design of companioned Android-based APP for interacting with the RAPID platform and communicating with other terminals. (a) Flowchart of the companioned Android-based APP: The APP connects the RAPID platform through Bluetooth to control testing and obtain results, where the results can also be transmitted to other kinds of (smart-mobile) devices by Bluetooth; It can also send the results to the hospital to obtain doctor's diagnosis and follow suggestions. (b) Log-in and results interface (along with recent history data, which presented by a curve of the target over time) of the Android-based APP.

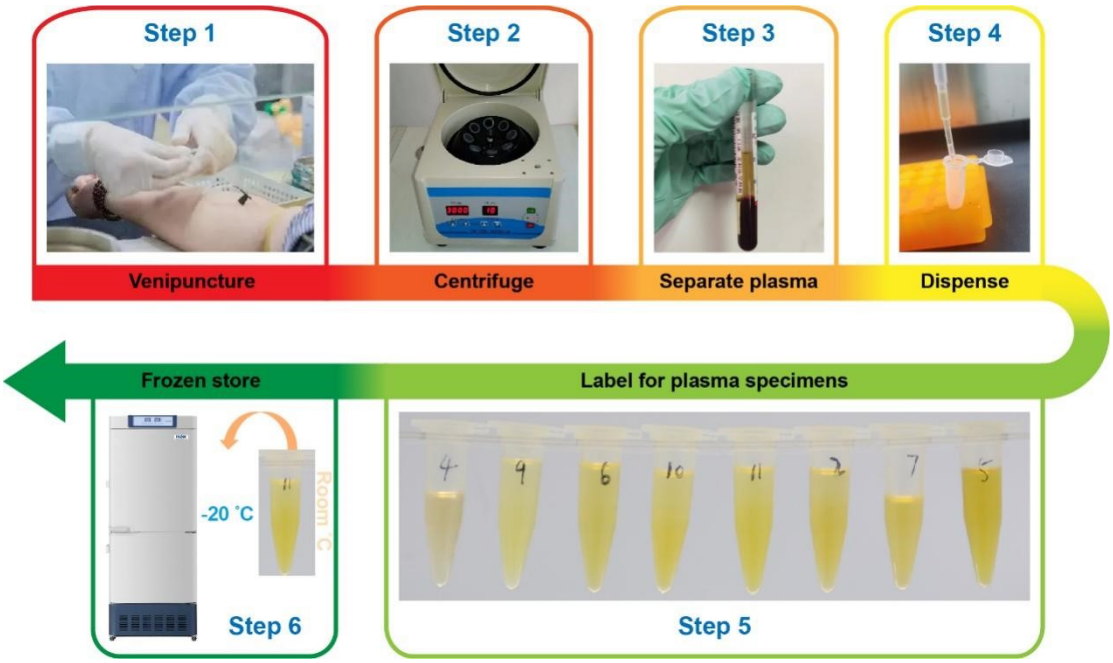

Figure S5. Results of an experiment evaluating interference of plasma background color to colorimetric assay.

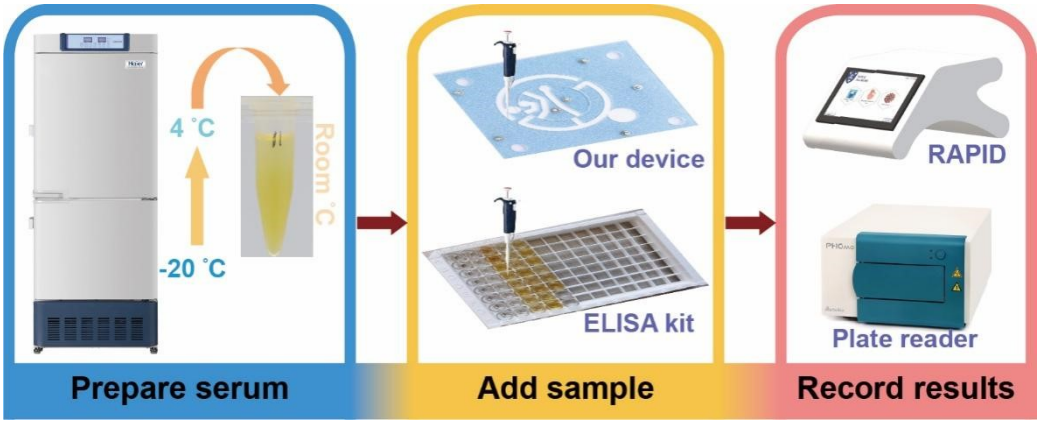

Figure S6. Illustration of steps for agreement analysis between the clinical performance of the RAPID and that of the gold standard commercial ELISA.

Table S1. Design and fabrication parameters of paper-based chip.

Design			**Fabrication**	
Overall dimensions	89 mm × 78 mm × 5 mm (L×W×H)		Wax printer	Xerox 8580DN
Valve diameter	34 mm		Laser cutter	Han's Yueming CMA0604-B-A
Channel width	2.8 mm		Laser parameter for paper	Intensity: 10% Speed: 50 mm/s
Overlap width	2 mm		Laser parameter for PMMA	Intensity: 40% Speed: 35 mm/s
Paper model	Whatman No. 1 chromatography paper		Hot plate	Cole-palmer 7"×7"
Flow rate (modified)	2.24 mm/s		Heat parameter	150 ℃, 60 s

Table S2. Design and fabrication parameters of RAPID platform.

Design			**Fabrication**	
Overall dimensions	179.66 × mm × 143 mm × 95.23 mm		3D printer	Ultimaker S5
Respond rate	76±0.6ms		Material	Ultimaker Tough PLA

Table S3. Models of electronic components used in the RAPID platform.

Component	Model
Battery module	Ywrobot
Servo motor of valve driver	Feetech SCS2332
Microcontroller	Arduino Uno R3
Bluetooth module	HM-10
Touch screen	Mzdesign ATF050
Illuminant screen	WaveShare 0.96 LCD
RGB sensor	ISL29125

Table S4. Raw data for results of specimens tested by RAPID and commercial ELISA.

Specimen number	Result of RAPID (pg)	Result of commercial ELISA (pg)
1	282.56	250.65
2	108.24	160.25
3	407.02	436.51
4	227.09	270.85
5	163.18	122.37
6	214.75	305.2
7	26.69	20.62
8	36.28	27.29
9	14.67	18.4
10	13.71	25.07
11	41.15	35.67
12	306.34	401.06
13	198.73	154.9
14	203.83	243.37
15	302.35	348.13
16	208.39	256.83
17	145.23	122.21
18	259.38	214.52
19	202.33	245.29
20	111.53	83.75
21	189.22	181.83
22	289.34	305.2
23	16.33	18.25
24	10.35	14.91

Table S5. One-test cost of the paper-based chip.

Item	Amount ($)
Paper, wax, PMMA, and screw/nut	0.41
Capture antibody	0.47
Blocking buffer	0.08
HRP-conjugated antibody	0.02
TMB substrate	0.004
PBS buffer	0.0001
Total	**0.98**

Table S6. Cost of the RAPID platform.

Item	Amount ($)
3D printing material	25.50
Electronic components	78.67
Other component/material	3.53
Total	**107.7**

Table S7. Comparison between our RAPID and the commercial ELISA.

	RAPID	Commercial ELISA
One-test cost	0.98 $	13.88 $
Instrument cost	107.7 $	Over 3000 $
Time to result	30 min	1.5 h
Sample volume	3 μL	100 μL
Portability	Yes	No
Capability of IOT	Yes	No
Sample-in-answer-out	Yes	No

ELISA is the most widely used analytical assay as it is the gold standard for detecting protein biomarkers in disease-related clinical samples, especially in low-resource settings [1-4]. This work mainly solves the problems of manual pipetting, low reproducibility, low efficiency, and the requirement for trained individuals to operate commercial ELISA. Meanwhile, it provides a feasible solution for early screening of AD. Therefore, we mainly compared the RAPID with commercial ELISA. In addition, commercial ELISA kits typically contain 96 wells (96 experiments). In contrast, our RAPID was the cost of one assay. Therefore, we divided the total reagent price of the commercial ELISA by 96 to obtain the price of a single assay. In addition, we tested 22 artificial plasma samples with RAPID and commercial ELISA. Among them, the commercial ELISA kit results were obtained from plate readers. The RAPID results were obtained from the platform's RGB sensor. Therefore, we also compared the prices of these two readout devices here.

References:

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