

# Progress, Application, and Strategic Priorities for zTB

## Diagnostic Capacity in Indonesia: Follow-Up Actions on Surveillance and Control Since the 2025 Training

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## IFN- $\gamma$ ELISA Training at the Serology Laboratory (July 2026)

### Principle of Ruminant IFN- $\gamma$ Test:

- **Mechanism:** Sandwich ELISA detecting IFN- $\gamma$  release from sensitized T-lymphocytes (Cell-Mediated Immunity).
- **Antigen Panel:** Evaluates differential stimulation between PPD-B (Bovine), PPD-A (Avian), PBS (Nil Control), and Mitogen (Viability Control).
- **Readout:** Absorbance measured at 450 nm; Cut-off threshold set at S/P  $\geq 15\%$  for positivity.



| Plate No         | 1         | 20260730-GB-IFNG/0614-000001-1 |        |           |        |
|------------------|-----------|--------------------------------|--------|-----------|--------|
| Reference        | Well Loc. | OD                             | Net OD | S/P Ratio | Result |
| Negative Control | A1        | 0,060                          |        |           |        |
| Negative Control | B1        | 0,056                          |        |           |        |
| Positive Control | C1        | 1,085                          |        |           |        |
| Positive Control | D1        | 0,861                          |        |           |        |
| PBS - 01         | E1        | 0,123                          |        | 13 %      | N      |
| PPDA - 01        | F1        | 0,168                          |        |           | P      |
| PPDB - 01        | G1        | 0,796                          | 0,628  | 69 %      | P      |
| Mitogen - 01     | H1        | 2,700                          |        | 295 %     | P      |
| PBS - 02         | A2        | 0,064                          |        | 7 %       | N      |
| PPDA - 02        | B2        | 0,065                          |        |           | N      |
| PPDB - 02        | C2        | 0,060                          | -0,005 | -1 %      | N      |
| Mitogen - 02     | D2        | 2,199                          |        | 240 %     | P      |
| PBS - 03         | E2        | 0,062                          |        | 7 %       | N      |
| PPDA - 03        | F2        | 0,115                          |        |           | P      |
| PPDB - 03        | G2        | 0,723                          | 0,608  | 66 %      | P      |
| Mitogen - 03     | H2        | 1,389                          |        | 152 %     | P      |
| PBS - 04         | A3        | 0,081                          |        | 9 %       | N      |
| PPDA - 04        | B3        | 0,090                          |        |           | P      |
| PPDB - 04        | C3        | 0,513                          | 0,423  | 46 %      | P      |
| Mitogen - 04     | D3        | 2,216                          |        | 242 %     | P      |
| PBS - 05         | E3        | 0,099                          |        | 11 %      | N      |
| PPDA - 05        | F3        | 0,114                          |        |           | N      |
| PPDB - 05        | G3        | 0,118                          | 0,003  |           | N      |
| Mitogen - 05     | H3        | 1,471                          |        | 161 %     | P      |
| PBS - 06         | A4        | 0,085                          |        | 9 %       | N      |
| PPDA - 06        | B4        | 0,424                          |        |           | N      |
| PPDB - 06        | C4        | 0,271                          | -0,153 | -17 %     | N      |
| Mitogen - 06     | D4        | 0,930                          |        | 102 %     | P      |
| PBS - 07         | E4        | 0,312                          |        | 34 %      | N      |
| PPDA - 07        | F4        | 1,160                          |        |           | P      |
| PPDB - 07        | G4        | 2,997                          | 1,837  | 201 %     | P      |
| Mitogen - 07     | H4        | 2,168                          |        | 237 %     | P      |
| PBS - 08         | A5        | 0,088                          |        | 10 %      | N      |
| PPDA - 08        | B5        | 0,127                          |        |           | P      |
| PPDB - 08        | C5        | 0,436                          | 0,308  | 34 %      | P      |
| Mitogen - 08     | D5        | 1,157                          |        | 126 %     | P      |
| PBS - 09         | E5        | 0,076                          |        | 8 %       | N      |
| PPDA - 09        | F5        | 0,526                          |        |           | P      |
| PPDB - 09        | G5        | 2,291                          | 1,765  | 193 %     | P      |
| Mitogen - 09     | H5        | 2,149                          |        | 235 %     | P      |

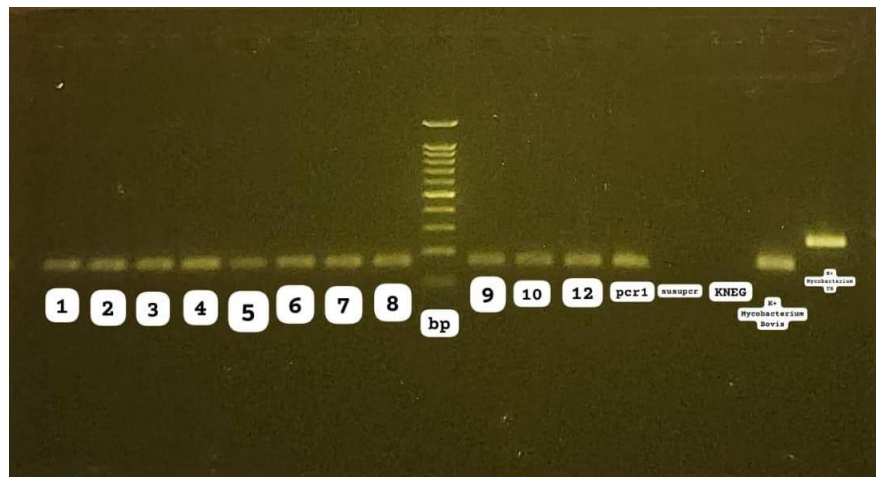


# Post-Training Follow-Up Actions

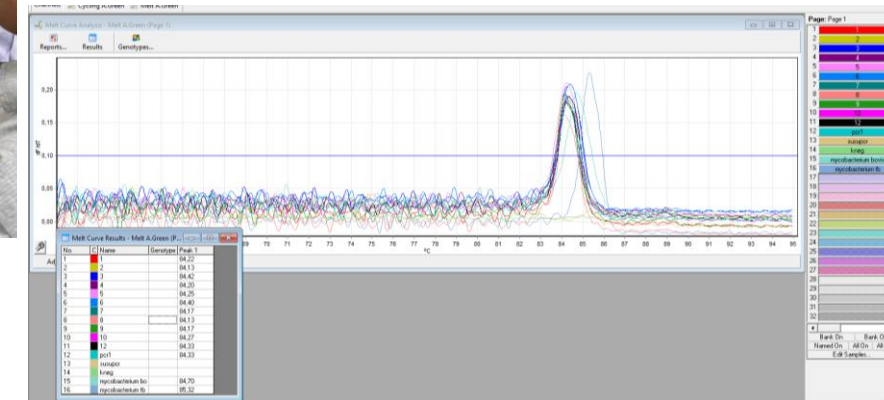
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## Tuberculosis Testing Training by BRIN Experts (October 2025)

1. **Training Overview:** *M. bovis* Laboratory Testing  
**Diagnostic Methods:** Agent identification via bacterial culture, Acid-fast staining (microscopy) and molecular detection (PCR).
2. **Sample Types Used:** Whole blood (and serum), probang sputum, milk, and organ tissues (lymph nodes and lungs).



Lanes 1–12 represent milk samples spiked with *Mycobacterium bovis*, all yielding positive *M. bovis* PCR bands (168 bp).

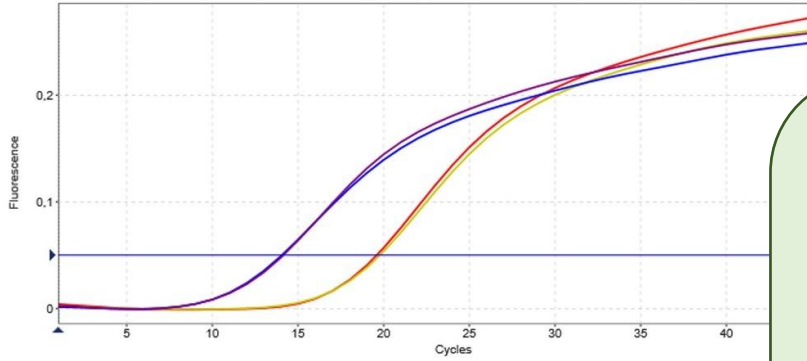


Single melting peak ( $T_m \approx 84,2^{\circ}\text{C}-84,7^{\circ}\text{C}$ ) was observed for *M.bovis* detection

# Application in Diagnostics and Surveillance

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Normalized Amplification Plot



## Molecular Testing (PCR) Execution:

- **Assay Method:** PCR testing using IS1081 and IS6110 primer sets.
- **Primer Source:** Brought by participants from DIC Subang, Indonesia, who attended the previous zTB Diagnostics Meeting in Qingdao last year.

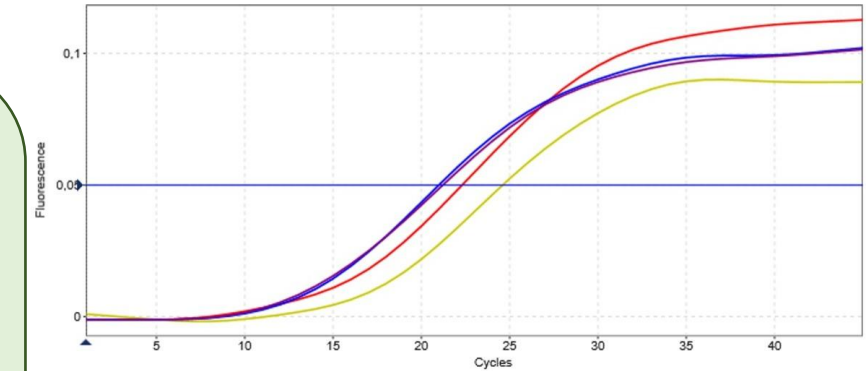
**Note:** TB = *M. tuberculosis*; BV) = *M. bovis*

Tube Results

| Tube        | Sample name | Sample groups | Sample type |      |  |
|-------------|-------------|---------------|-------------|------|--|
| Acquisition | Target      | Cq            | Take-off    | Eff. |  |
| 1           | TB          |               | PC          |      |  |
| Green       | Page 1      | 19,60         | 13,90       | 1,07 |  |
| 2           | TB          |               | PC          |      |  |
| Green       | Page 1      | 19,78         | 13,90       | 1,50 |  |
| 3           | BV          |               | PC          |      |  |
| Green       | Page 1      | 14,06         | 7,10        | 1,39 |  |
| 4           | BV          |               | PC          |      |  |
| Green       | Page 1      | 14,15         | 7,00        | 1,36 |  |

**IS1081 Primers for MTBC Detection**

Normalized Amplification Plot



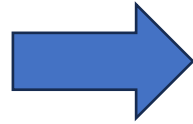
Tube Results

| Tube        | Sample name | Sample groups | Sample type |      |  |
|-------------|-------------|---------------|-------------|------|--|
| Acquisition | Target      | Cq            | Take-off    | Eff. |  |
| 1           | TB          |               | PC          |      |  |
| Green       | Page 1      | 22,30         | 7,20        | 0,12 |  |
| 2           | TB          |               | PC          |      |  |
| Green       | Page 1      | 24,59         | 13,00       | 0,19 |  |
| 3           | BV          |               | PC          |      |  |
| Green       | Page 1      | 20,98         | 7,60        | 0,03 |  |
| 4           | BV          |               | PC          |      |  |
| Green       | Page 1      | 21,21         | 6,90        | 0,00 |  |

**IS6110 Primers for MTBC Detection**

## Case Chronology in DIC Subang Working Area

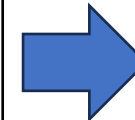
**30 July 2026: Diagnostic testing showed Positive results on *M. bovis* IGRA ELISA and Seropositive on MTb Antibody ELISA**



**04 August 2026 (04:30 WIB): Found collapsed/recumbent by personnel at Subang Disease Investigation Center (DIC Subang)**

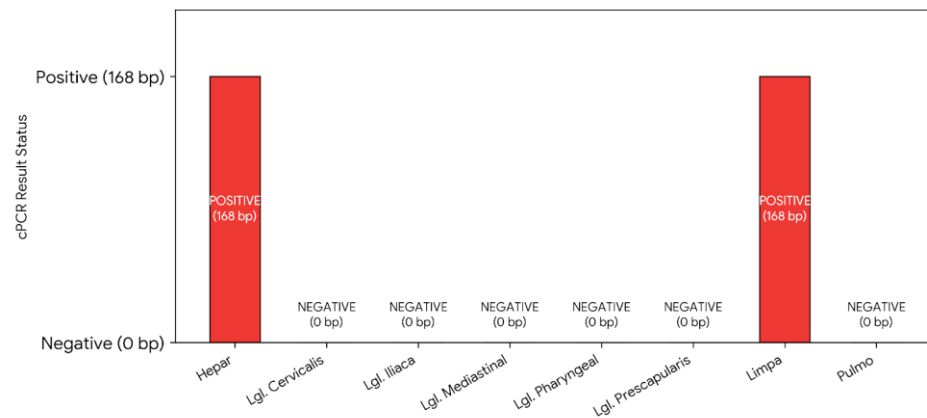


**04 August 2026 (06:00 WIB)  
Clinical Signs: Respiratory distress (dyspnea) and subnormal pulse rate**



**04 August 2026 (08:00 WIB) :  
Outcome: Planned for culling following test results, but emergency slaughter was performed due to acute collapse.**

M. bovis cPCR Detection by Organ/Sample Type



## Bovine Necropsy Report

### Detailed Gross Lesion Findings in Lungs

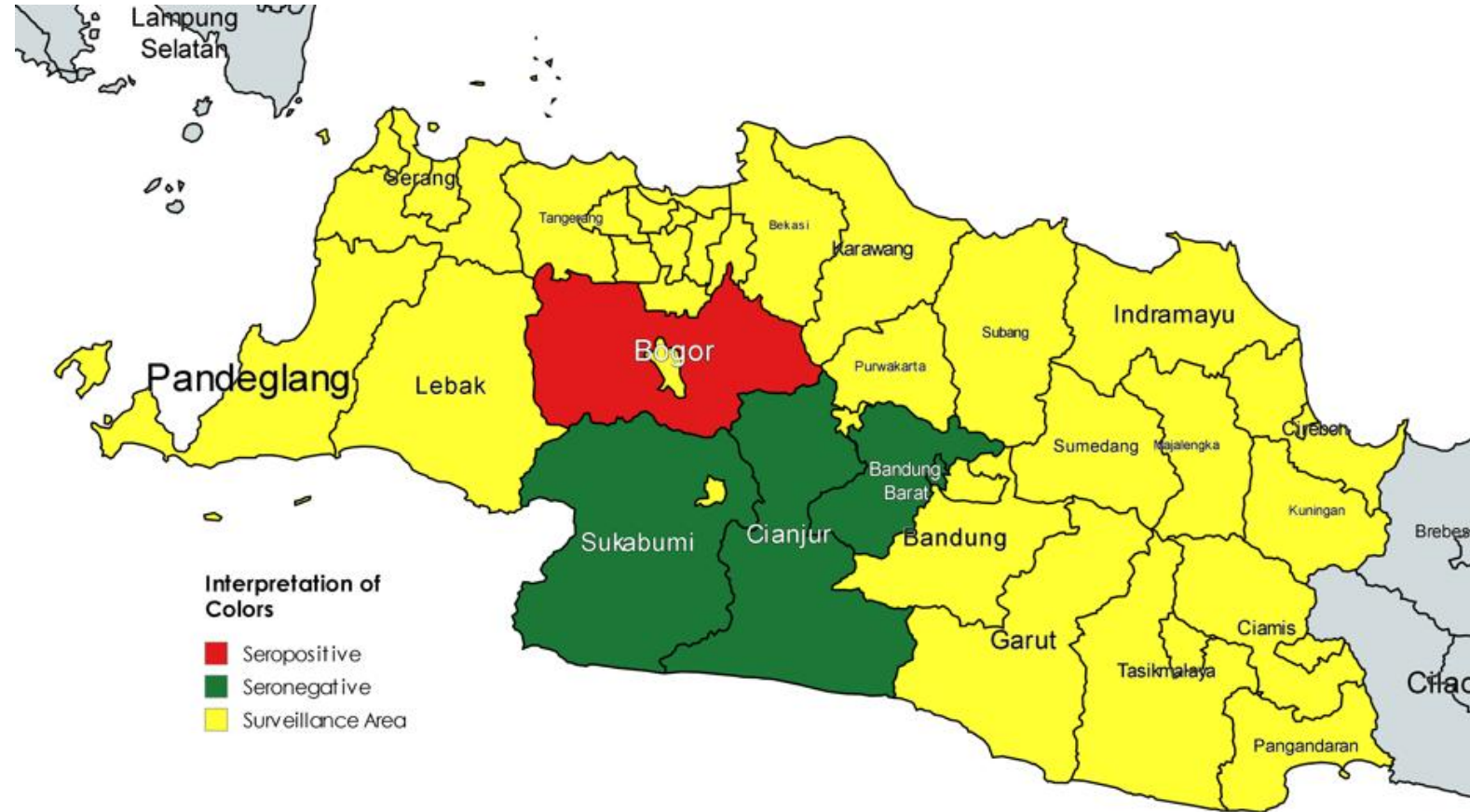
- **Gross Pathology:** Cross-sections of the lung parenchyma reveal thick, yellowish-white caseous material within the pulmonary tissue and regional (peribronchial/mediastinal) lymph nodes.
- **Diagnostic Significance:** These lesions are pathognomonic features of bovine tuberculosis, typically characterized by caseous granulomas or tubercles within the lungs and draining lymph nodes.





## 3. Diagnostic Elisa Antibody For ZTB in DIC Subang In 2026

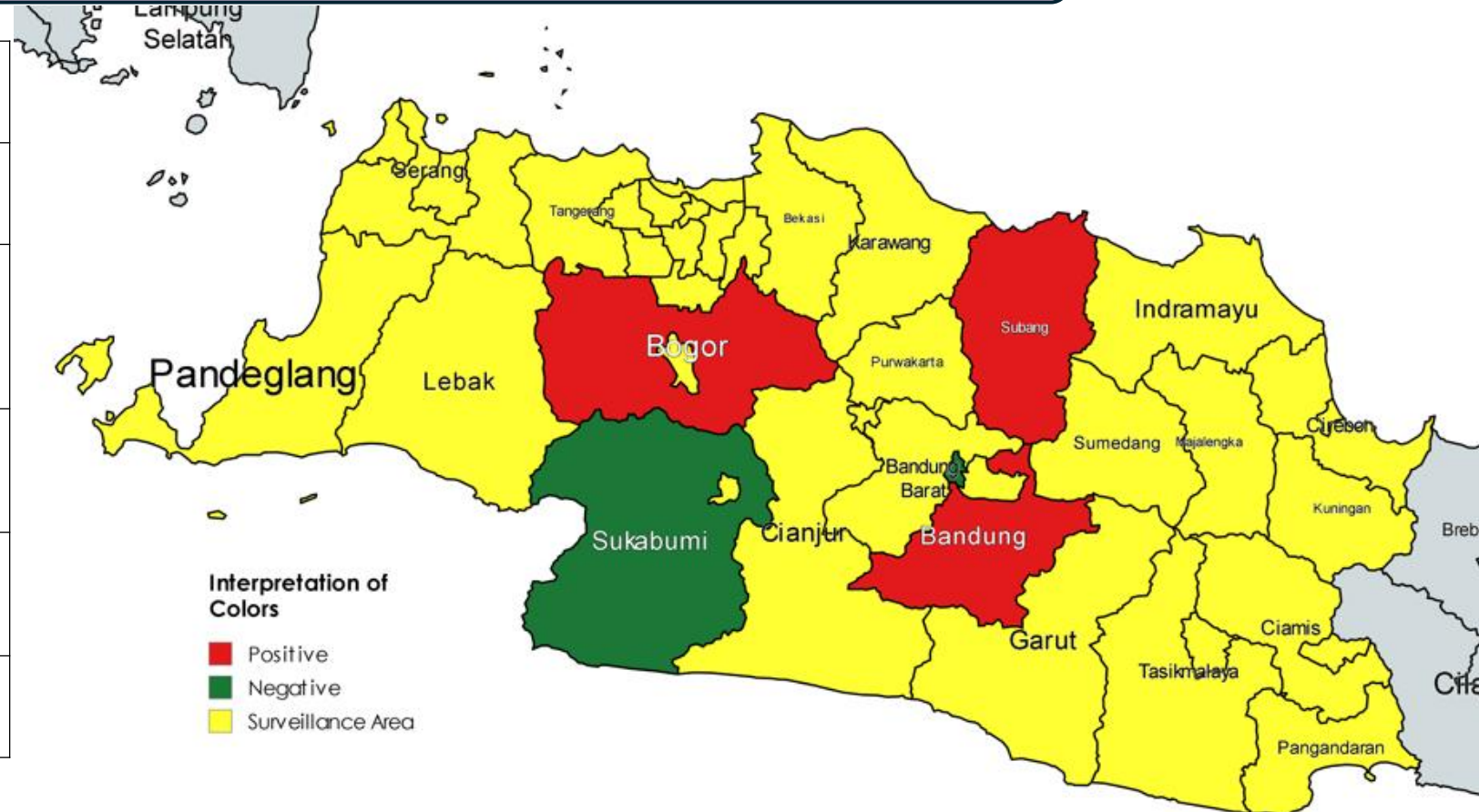
| No | District      | sample | Animal | Total sample | Result                                 |
|----|---------------|--------|--------|--------------|----------------------------------------|
| 1  | Bogor         | Serum  | Cow    | 24           | Seronegative (9),<br>Seropositive (15) |
| 2  | Cianjur       | Serum  | Cow    | 13           | Seronegative (13)                      |
| 3  | Sukabumi      | Serum  | Cow    | 20           | Seronegative (20)                      |
| 4  | Bandung Barat | Serum  | Cow    | 13           | Seronegative (13)                      |





## 4. Diagnostic PCR For ZTB in DIC Subang In 2026

| No | District    | sample                | Animal | Total sample | Result                       |
|----|-------------|-----------------------|--------|--------------|------------------------------|
| 1  | Kota Cimahi | Swab                  | Cow    | 1            | Negative (1)                 |
| 2  | Subang      | Organ, swab and blood | Cow    | 48           | Positive (11); negative (37) |
| 3  | Bandung     | Swab and organ        | Cow    | 30           | Positive (5); negative (25)  |
| 4  | Bogor       | Swab and organ        | Cow    | 65           | Positive (1); negative (64)  |
| 5  | Sukabumi    | Swab                  | Cow    | 37           | Negative (37)                |





# Future Priorities and Resource Needs

There is no routine surveillance for TB in animal.

- Conducting surveillance and monitoring of ZTB in Indonesia continuously
- Completing media and laboratory equipment for ZTB (At least BSL 3 is required in the reference DIC)
- Need for a ZTB reference laboratory, especially DIC in Indonesia
- Improving human resource capabilities
- Improving one health collaboration

All these steps become the target for the future so that Ztb in Indonesia can be addressed and all involved agencies can collaborate well.

# Thank you!

## Contact information

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