

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(9): 524-530
www.biochemjournal.com
Received: 01-07-2026
Accepted: 04-08-2026

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Multi-trait evaluation and selection of superior *Apis mellifera* L. colonies based on brood survival, hygienic behaviour and colony productivity

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9g.10049>

Abstract

The study was conducted at All India Coordinated Research Project on Honeybee and Pollinator, Department of Entomology, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India and evaluated ten colonies using brood survival, honey and pollen stores and hygienic behaviour, with the objective of identifying colonies suitable for further breeding and apiary multiplication. Ten *A. mellifera* colonies were assessed during 2024. Brood survival was estimated in a marked 25 cm² area by recording eggs laid, eggs hatched and surviving larvae. Honey and pollen stores were quantified from frame units using the conversion factors provided in the experimental records. Hygienic behaviour was evaluated from weekly debris removal and by the removal of 25 experimentally killed pupae. A derived multi-trait index was calculated by min-max standardization of five traits, with debris removal treated as a positive criterion after inverse scaling. Mean brood survival was 90.91%, ranging from 77.42% in C₉ to 97.06% in C₅. Honey stores ranged from 257.92 to 403.00 g and pollen stores from 25.80 to 37.41 cm². C₅ had the highest brood survival, honey and pollen stores and removed all experimentally killed pupae (100%). C₃ also showed strong performance, with 93.75% brood survival and 96% pupal removal. C₉ showed the weakest overall performance and the highest debris accumulation. The derived multi-trait ranking placed C₅ first, followed by C₃ and C₆. The results demonstrate substantial colony-to-colony variation in traits relevant to productivity and hygienic defence. C₅ was the most promising colony under the present dataset and could be prioritized for further validation as a breeding colony. Multi-trait selection is preferable to selection based on a single colony characteristic.

Keywords: *Apis mellifera*, brood survival, colony productivity, hygienic behaviour, honey stores, pollen stores, selection

Introduction

Honey bees are important pollinators and provide economically valuable hive products. In managed *Apis mellifera* populations, colony productivity is determined by interacting traits including worker population, queen performance, brood production, food reserves, foraging activity and behavioural resistance to pests and pathogens. Indian studies have demonstrated substantial variation among colonies in brood area, honey reserves, pollen stores, brood survival and other desirable traits, providing a basis for selection of superior colonies (Yadav *et al.*, 2023; Sharma *et al.*, 2020; Brar *et al.*, 2018; Saini *et al.*, 2018; Saini *et al.*, 2018a) [30, 19, 3, 17, 18].

Brood survival is an important indicator of colony health because it integrates egg viability, larval care, food availability and the ability of worker bees to maintain developing brood. Colony strength and brood production are also closely related to honey and pollen reserves, particularly during periods of intensive colony development and honey flow (Kumar *et al.*, 2024; Pande *et al.*, 2011; Nadaf *et al.*, 2020; Chhuneja *et al.*, 1992) [8, 15, 13, 6]. Selection based on several traits simultaneously can therefore identify colonies that are both productive and biologically robust.

Hygienic behaviour is another important component of colony defence. Hygienic workers detect abnormal or dead brood, uncap affected cells and remove the brood, thereby limiting the persistence and spread of brood diseases and some parasites (Singh *et al.*, 2007; Rana and Sharma 2017; Sharma *et al.*, 2017; Kumar and Kumar 2014) [22, 16, 20]. Indian screening programmes have demonstrated substantial variability in hygienic behaviour among *A.*

mellifera colonies and have emphasized its potential as a selection criterion for breeding programmes (Srivastava *et al.*, 2017; Wahid and Singh 2025; Nath *et al.*, 2024) [27, 28, 14]. The litchi-growing region of Bihar is particularly relevant to managed *A. mellifera* because litchi orchards provide an important nectar and pollen resource during bloom. Studies at the ICAR-National Research Centre on Litchi, Muzaffarpur, have shown that *A. mellifera* is an important litchi pollinator and that pollination by honey bees can influence fruit set, yield and quality (Mahindru *et al.*, 2021; Mahindru *et al.*, 2023) [12, 11]. Recent work has further characterized *A. mellifera* pollen-foraging dynamics during litchi bloom in Muzaffarpur (Kaur *et al.*, 2025) [7]. Thus, identifying robust colonies is relevant not only to hive productivity but also to pollination-service management. Although several Indian investigations have evaluated individual colony traits, fewer studies combine brood survival, food reserves and hygienic behaviour into a single selection framework. Therefore, the present study evaluated ten *A. mellifera* colonies using these complementary traits and developed a derived multi-trait ranking to identify promising colonies for future breeding and validation.

2. Materials and Methods

2.1 Experimental colonies and study period

The study involved ten *Apis mellifera* L. colonies maintained at the AICRP on Honeybee and Pollinators, Department of Entomology, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India during litchi crop bloom in 2024. Observations supplied in the experimental record were collected during 2024. Colonies were designated C₁-C₁₀. The present manuscript analyses the supplied colony-level observations without changing the original values.

2.2 Brood survival and brood pattern

A marked area of 25 cm² was used for brood assessment. The numbers of eggs laid, eggs hatched and surviving larvae were recorded. Brood survival percentage was calculated from the experimental observations as the proportion of surviving brood relative to eggs laid. Colonies were classified as high, medium or low according to the supplied categories, and brood continuity was scored on the recorded 1-3 scale.

2.3 Honey and pollen stores

Honey stores were estimated from the number of squares filled with honey using the supplied conversion factor of 8.06 g per square. Pollen storage was recorded from pollen-filled cells and grid squares; pollen store area was calculated using the supplied factor of 6.45 cm² per grid square. These calculations were retained exactly as provided in the source dataset.

2.4 Hygienic behaviour

Two complementary indicators were used. First, the quantity of debris removed from each hive was recorded weekly on 24 March, 1 April, 8 April and 15 April 2024, and the mean was used to characterize nest-cleaning activity. Second, 25 pupae were experimentally killed in each colony and the number subsequently removed was recorded. Removal percentage was calculated as removed pupae divided by 25 × 100.

2.5 Multi-trait selection index

To provide a selection-oriented interpretation, five traits were standardized using min-max scaling across the ten colonies: brood survival, honey stores, pollen stores, pupal-removal percentage and debris removal. For debris, lower accumulation was treated as favourable and therefore reverse-scaled. The unweighted multi-trait index was calculated as the arithmetic mean of the five standardized scores. This derived index is an analytical ranking of the supplied data and is not an additional field observation.

2.6 Statistical presentation

The supplied dataset included means, standard errors, critical differences and coefficients of variation where reported. Results are presented descriptively by colony, followed by the derived multi-trait ranking. The manuscript does not claim an additional experimental replication or inferential test beyond the statistics contained in the supplied tables.

3. Results

3.1 Brood survival and brood continuity

The ten colonies differed in brood survival. The overall mean was 90.91%. C₅ recorded the highest brood survival (97.06%), followed by C₃ (93.75%), C₆ (93.48%), C₈ (93.18%) and C₁₀ (93.02%). C₉ recorded the lowest value (77.42%) and was categorized as low brood survival. C₁ was also categorized as high (92.86%), whereas C₂, C₄ and C₇ were classified as medium. Seven colonies had a brood-continuity score of 1, three had score 2, and C₉ had score 3 in Table 1 and Fig. 1A.

3.2 Colony-wise honey and pollen reserves

Food reserves varied substantially among colonies. C₅ had the largest honey reserve (403.00 g) and pollen store (37.41 cm²). C₃ ranked next for honey (386.88 g) and had 32.90 cm² pollen stores. C₆ had 370.76 g honey and 34.83 cm² pollen. C₉ recorded the lowest honey (257.92 g) and pollen (25.80 cm²) stores. The overall means were 340.13 g honey and 31.28 cm² pollen in Table 2 and Fig. 1B and C.

3.3 Hygienic behaviour based on debris removal

Mean debris removal/cleaning values ranged from 1.28 g in C₅ to 2.77 g in C₉. C₃ (1.64 g) and C₁₀ (1.73 g) also showed low debris accumulation, while C₉ showed the highest value and was classified as poor cleaning. C₅ and C₃ were classified as very clean; the remaining colonies except C₉ were classified as clean presented in Table 3 and Fig. 1 D.

3.4 Removal of experimentally killed pupae

The mean removal of experimentally killed pupae was 91.60%. C₅ removed all 25 killed pupae (100%), while C₃, C₆ and C₈ each removed 24 pupae (96%). C₁, C₇ and C₁₀ removed 23 pupae (92%), whereas C₂ and C₄ removed 22 (88%). C₉ removed 19 pupae, corresponding to 76%, the lowest value in Table 4 and Fig. 1 E.

3.5 Multi-trait selection and identification of superior colonies

The derived multi-trait index integrated brood survival, honey, pollen, pupal removal and inverse debris accumulation. C₅ was ranked first because it simultaneously expressed the highest brood survival, the highest honey and pollen stores, the lowest debris accumulation and complete

removal of killed pupae. C₃ and C₆ followed, reflecting consistently strong values across most traits. C₉ ranked last because it combined low brood survival, low honey and pollen stores, high debris accumulation and the lowest pupal-removal percentage.

4. Discussion

The present observations demonstrate clear colony-level variation in brood viability and resource accumulation. Such variation is expected in managed *A. mellifera* populations and is the basis for selecting breeding colonies rather than treating the apiary as a homogeneous population. Indian selection studies have similarly reported significant differences among colonies in bee strength, brood area, honey reserves, pollen reserves and brood survival, and have recommended ranking colonies across several traits rather than relying on a single measure colonies (Yadav *et al.*, 2023; Sharma *et al.*, 2020; Brar *et al.*, 2018; Saini *et al.*, 2018; Saini *et al.*, 2018a)^[30, 19, 3, 17, 18].

C₅ was consistently superior in the present dataset. Its brood survival of 97.06% was the highest, while its honey and pollen stores were also maximal (Singh *et al.*, 2019; Sharma *et al.*, 2018; Chaudhary *et al.*, 2020; Lalita *et al.*, 2025; Bawa and Marwaha 2026)^[23, 21, 5, 10, 2]. This pattern agrees with Indian studies showing that stronger and better-performing colonies generally maintain greater brood and food reserves (Kumar *et al.*, 2024; Pande *et al.*, 2011; Nadaf *et al.*, 2020; Chhuneja *et al.*, 1992)^[8, 15, 13, 6]. However, the present results should be interpreted as colony-level association rather than proof that one trait directly caused another.

The hygienic results provide an additional basis for selection. C₅ removed 100% of the experimentally killed pupae and had the lowest mean debris value. C₃ also showed high hygienic performance. Hygienic behaviour is widely recognized as a colony-level defence involving recognition, uncapping and removal of abnormal brood (Chatterjee *et al.*, 2025; Singh *et al.*, 2014; Spivak and Gilliam 1998; Woyke *et al.*, 2004; Arathi *et al.*, 2000; Spivak and Danka 2021)^[4, 24, 26, 29, 1, 25]. Indian screening studies have shown

considerable variation in this trait and have emphasized its usefulness in breeding programmes (Srivastava *et al.*, 2017; Wahid and Singh 2025; Nath *et al.*, 2024)^[27, 28, 14].

The strong performance of C₅ across productivity and hygiene is particularly valuable from a breeding perspective because selection based only on honey yield can overlook health-related traits. Conversely, selecting only for hygienic behaviour could favour colonies that are behaviourally resistant but not necessarily highly productive. A multi-trait approach reduces this risk and is consistent with Indian colony-selection programmes that use composite scoring systems (Yadav *et al.*, 2023; Saini *et al.*, 2018; Saini *et al.*, 2018a)^[30, 17, 18].

The litchi context is also important for the broader application of superior colonies in Bihar. Litchi is a major horticultural crop in the region, and studies from Muzaffarpur have documented *A. mellifera* as an important flower visitor and pollinator. Kumar and Kumar (2014)^[9] reported that *A. mellifera* was the dominant recorded forager in their litchi observations and that proximity to colonies was associated with higher fruit set and yield (Mahindru *et al.*, 2021)^[12]. Srivastava *et al.* (2017)^[27] documented a diverse pollinator complex and high *A. mellifera* activity on litchi flowers Mahindru *et al.*, 2023)^[11]. Recent pollen-foraging research during litchi bloom has further demonstrated the importance of *A. mellifera* colonies for understanding pollen resources in Muzaffarpur orchards (Kaur *et al.*, 2025)^[7]. These findings support the practical importance of maintaining strong, healthy colonies for pollination services.

Nevertheless, the present selection index is a derived analytical tool based on one supplied dataset. It should not be interpreted as a finalized breeding value. Before C₅ or other colonies are used extensively as breeder colonies, their performance should be repeated over multiple seasons and locations, with queen age/genetic background, colony strength, disease and parasite load, honey yield, brood pattern and hygienic behaviour assessed under standardized conditions.

Table 1: Assessment of brood survival rate and scattered brood pattern in *Apis mellifera* colonies during 2024

Colony no.	No. of eggs laid in marked area (25 Sq.cm)	No. of eggs hatched	Larvae survived	Brood survival rate (%)	Category	Score based on brood continuity pattern
C ₁	42	41	39	92.86	High brood survival rate	1
C ₂	35	34	31	88.57	Medium brood survival rate	2
C ₃	48	47	45	93.75	High brood survival rate	1
C ₄	40	39	36	90.00	Medium brood survival rate	2
C ₅	34	34	33	97.06	High brood survival rate	1
C ₆	46	45	43	93.48	High brood survival rate	1
C ₇	39	38	35	89.74	Medium brood survival rate	2
C ₈	44	43	41	93.18	High brood survival rate	1
C ₉	31	30	24	77.42	Low brood survival rate	3
C ₁₀	43	42	40	93.02	High brood survival rate	1
Mean	40.20	39.30	36.70	90.91		
SEm±	1.74	1.70	1.98	1.68		
CD (< 5%)	3.87	3.79	4.42	3.75		
CV (%)	13.66	13.68	17.09	5.86		

Table 2: Colony-wise variation in brood survival rate, honey stores and pollen stores in *Apis mellifera* L. colonies during 2024

Colony Number	Brood survival rate (%)	No. of square filled with honey	Quantity of honey (g) (No of square filled with honey $\times$ 8.06)	No. of cells filled with pollen	No. of squares in grid frame covered with pollen	Pollen stores (cm ²) (No. of squares in grid frame covered with pollen $\times$ 6.45)
C ₁	92.86	42.00	338.52	43.00	4.30	27.74
C ₂	88.57	35.00	282.10	47.00	4.70	30.32
C ₃	93.75	48.00	386.88	51.00	5.10	32.90
C ₄	90.00	41.00	330.46	45.00	4.50	29.03
C ₅	97.06	50.00	403.00	58.00	5.80	37.41
C ₆	93.48	46.00	370.76	54.00	5.40	34.83
C ₇	89.74	39.00	314.34	46.00	4.60	29.67
C ₈	93.18	45.00	362.70	53.00	5.30	34.19
C ₉	77.42	32.00	257.92	40.00	4.00	25.80
C ₁₀	93.02	44.00	354.64	48.00	4.80	30.96
Mean	90.91	42.20	340.13	48.50	4.85	31.28
SE $\pm$	1.69	1.79	14.41	1.73	0.17	1.12
CV (%)	5.86	13.40	13.40	11.30	11.30	11.30

Table 3: Evaluation of hygienic behaviour of *Apis mellifera* L. colonies based on weight of debris removed from the hive (g)

Colony Number	24.03.2024	01.04.2024	08.04.2024	15.04.2024	Mean	Status
C ₁	1.72	1.84	1.76	2.21	1.88	Clean
C ₂	1.65	1.78	1.82	2.14	1.85	Clean
C ₃	1.48	1.55	1.62	1.91	1.64	Very clean
C ₄	1.93	2.04	2.11	2.48	2.14	Clean
C ₅	1.18	1.27	1.22	1.46	1.28	Very clean
C ₆	1.57	1.69	1.73	2.06	1.76	Clean
C ₇	1.86	2.13	2.04	2.29	2.08	Clean
C ₈	1.51	1.67	1.59	2.18	1.74	Clean
C ₉	2.34	2.71	2.86	3.18	2.77	Poor cleaning
C ₁₀	1.69	1.58	1.71	1.93	1.73	Clean
Mean	1.69	1.83	1.85	2.18	1.88	
SEm $\pm$	0.10	0.12	0.12	0.14	0.09	
CD (< 5%)	0.30	0.35	0.36	0.42	0.28	
CV (%)	12.82	10.77	10.54	12.12	14.16	

Table 4: Hygienic behaviour of *Apis mellifera* L. colonies based on removal of experimentally killed pupae

Colony no.	Per cent brood survival	Pupae killed	Removed pupae (dead)	Removal (%)
C ₁	92.86	25.00	23.00	92.00
C ₂	88.57	25.00	22.00	88.00
C ₃	93.75	25.00	24.00	96.00
C ₄	90.00	25.00	22.00	88.00
C ₅	97.06	25.00	25.00	100.00
C ₆	93.48	25.00	24.00	96.00
C ₇	89.74	25.00	23.00	92.00
C ₈	93.18	25.00	24.00	96.00
C ₉	77.42	25.00	19.00	76.00
C ₁₀	93.02	25.00	23.00	92.00
Mean	90.91	25.00	22.90	91.60
SE $\pm$	1.69	0.00	0.53	2.10
CV (%)	3.75	0.00	1.17	4.68

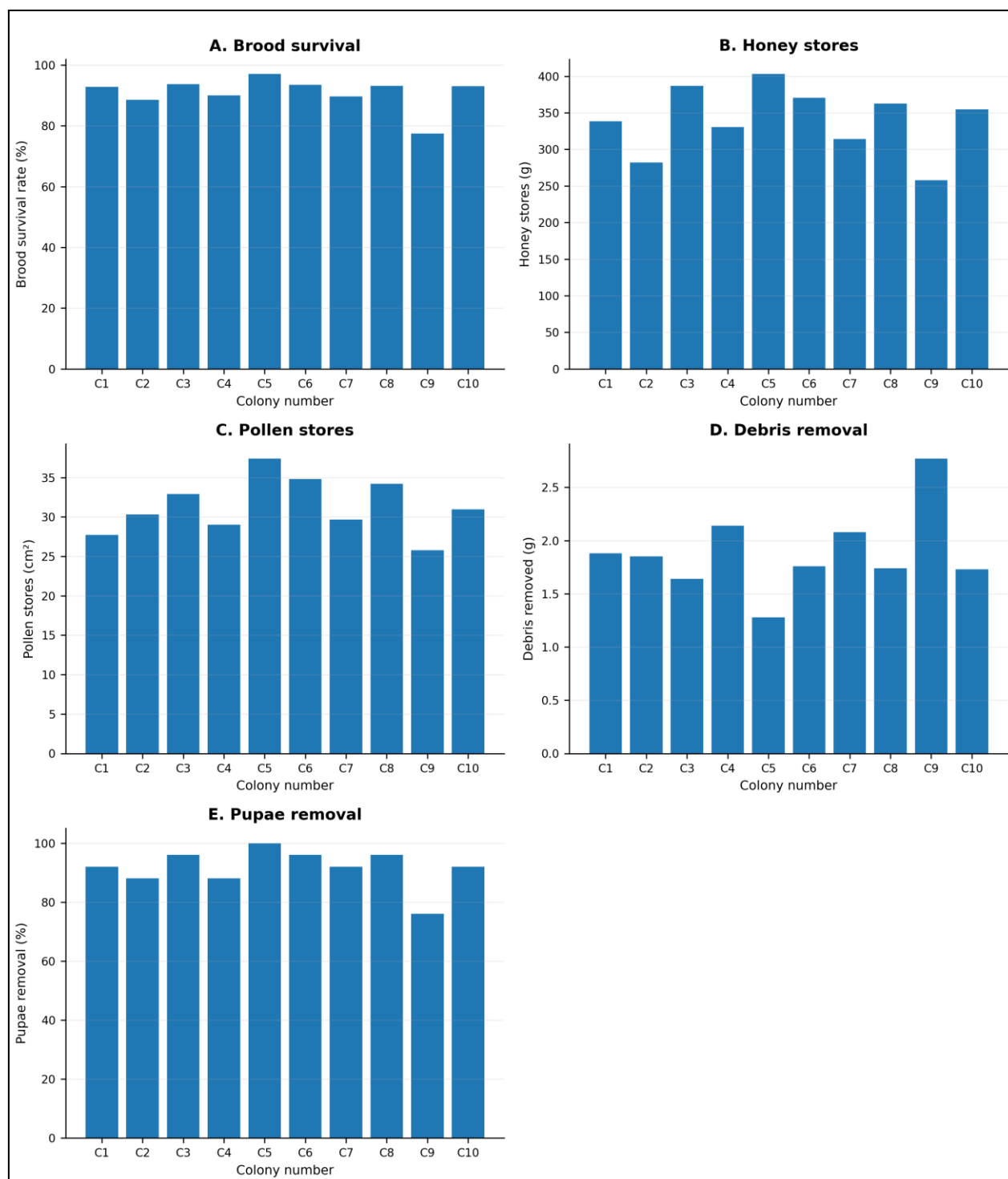


Fig 1: Colony wise variation in brood performance, stores and hygienic behaviour of *Apis mellifera*

5. Conclusion

Ten *Apis mellifera* colonies showed substantial variation in brood survival, honey and pollen reserves and hygienic behavior on litchi crop. C₅ was the best-performing colony in the supplied dataset, recording 97.06% brood survival, 403.00 g honey, 37.41 cm² pollen stores, the lowest debris accumulation (1.28 g) and 100% removal of experimentally killed pupae. C₃ and C₆ were additional promising colonies. C₉ consistently displayed inferior values and ranked last in the derived multi-trait index. The study supports multi-trait colony evaluation as a practical approach for identifying candidate colonies for further breeding and apiary multiplication. Repeated evaluation across seasons and

floral environments is recommended before definitive genetic selection.

6. Acknowledgement

The authors acknowledge the AICRP on Honeybee and Pollinators and the Department of Entomology, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, for the facilities and support associated with the honeybee observations.

7. Conflict of Interest

The authors declare that they have no conflict of interest.

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