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GHIC POSITION PAPERS

Proteomics in honey control

From protein identification to effective, proportionate enforcement

Executive summary

Proteomics can identify protein targets that an enzyme-activity measurement alone cannot distinguish. This gives honey control an additional means of investigating industrial-enzyme inputs, atypical protein patterns and some consequences of feeding or processing. GHIC supports prompt, evidence-based use of these findings in supply-chain investigations and official controls.

> **GHIC position:** A well-confirmed, relevant foreign-protein finding cannot be dismissed because another limited panel is negative. Its identity, plausible route into the product and legal significance must be assessed explicitly.

Detecting a protein, establishing non-compliance and proving intentional fraud are three different conclusions. The presence of a peptide assigned to a microbial enzyme does not, without further evidence, identify its manufacturer, the stage of entry or a responsible person. Equally, hypothetical natural explanations should be investigated rather than used automatically to set aside a strong analytical result.

In the supplied GHIC pair, D339H had two foreign enzyme targets reported positive, atypical bee-protein findings and a non-authentic DNA classification. D340H was unremarkable for the protein parameters examined and was classified authentic by DNA. These are mutually compatible findings in two samples, not a prevalence study. [7]

- Confirm molecular identity using a validated, matrix-appropriate workflow.
- Examine industrial addition, feed carry-over, natural microbial sources, processing and contamination against the specific marker.
- Preserve the lot and counter-sample evidence; use proportionate, legally grounded measures when the evidential threshold is met.

The objective is an effective control system that can withstand technical challenge and protect authentic honey.

1 · Protein identity and enzyme activity answer different questions

Diastase activity measures starch breakdown under assay conditions. It does not identify the enzyme responsible. Proteomics can identify characteristic peptide sequences after protein extraction and digestion, usually by liquid chromatography with tandem or high-resolution mass spectrometry. A normal activity value cannot, on its own, establish that the activity is of the expected origin. [3, 8]

Measurement	What it supports	What it does not establish alone
Enzyme activity	Catalytic activity under specified conditions	Species or production source of the enzyme
Targeted peptide identification	Presence of a defined protein target within method capability	Amount of added syrup, time of addition or intent
Bee-protein panel	Measured status of selected bee proteins	A universal genuine-honey guarantee or an automatic dilution diagnosis
Untargeted proteome	Broader discovery of protein patterns and candidate markers	Validated performance of every discovered marker

The documented targeted panel

The Eurofins provider description covers seven foreign targets: α-amylases assigned to Aspergillus oryzae, Bacillus licheniformis and B. amyloliquefaciens; barley β-amylase; Aspergillus niger amyloglucosidase; Rhizopus glucoamylase; and Saccharomyces cerevisiae invertase. It also assesses MRJP1 and two bee enzymes. [8]

Invertase acts on sucrose; it should not be described as directly restoring the diastase result. “Foreign” is an analytical classification and is not synonymous with “synthetic”: industrial preparations often contain proteins produced by naturally occurring organisms.

The provider describes qualitative detection in the low µg/g range, depending on the target and equipment. This is not a universal syrup-percentage detection limit. A negative result covers the stated panel and detection capability, not all possible adulterants.

2 · What the current research adds

Proof of analytical capability

Erban and colleagues (2021) reported foreign amylases in four of 45 investigated honeys using proteomic analysis. The study supports molecular identification of specific foreign enzymes; the sample set does not establish a global prevalence. [3]

Takahashi and colleagues (2023) examined 67 commercial honeys with an improved native-PAGE activity-staining method and compared findings with LC-QTOF-MS. They reported Aspergillus amylases in 13 samples and Geobacillus amylases in six. These counts should not be treated as a universal panel sensitivity or as evidence that activity staining covers all foreign proteins. [4]

Broader proteins and possible entry routes

Erban and colleagues (2026) studied 29 honeys and a royal-jelly control and reanalysed an earlier 45-honey dataset. The reported findings include enzymes associated with syrup or fondant manufacture and soybean proteins. The authors discuss feeding practices as well as adulteration-related explanations. Protein origin and route of entry therefore need separate investigation. [5]

Natural microbiology must be tested marker by marker

Wang and colleagues (2025) investigated Bacillus subtilis as a contributor to amylase production in the honey sac of Apis mellifera. This is relevant evidence against assuming that every microbial amylase necessarily proves industrial addition. It does not demonstrate that the A. oryzae and S. cerevisiae targets in D339H arose naturally, nor does it invalidate their analytical detection. [6, 7]

> GHIC requires the same evidential discipline in both directions: do not infer intentional addition merely from the word “microbial”, and do not invoke a natural source without examining whether it explains the specific sequence, level, co-markers and production history.

3 · The supplied orthogonal case pair

The controlling identifiers below reconcile the Celvia and Eurofins records. Internal shorthand labels in the underlying documents differ; identifiers and lot links are more reliable than a repeated “BG” label. The reported classifications are reproduced as analytical outcomes, not GHIC-issued certificates. [7]

Reported parameter	D339H	D340H
Eurofins sample	296-2026-00011503	296-2026-00011501
Lot	L25032026-1	L2207 / 2207
Celvia MDA v2, 10.07.2026	NON-AUTHENTIC	AUTHENTIC
Raw sequences / studied objects	10,059,764 / 273	3,627,744 / 284
α-amylase: A. oryzae	Positive	Negative
Invertase: S. cerevisiae	Positive	Negative
Other five foreign targets	Negative	Negative
MRJP1	LOW	PASSED
Bee α-amylase / α-glucosidase	ABSENT / ABSENT	PASSED / PASSED
Protein report, 23.07.2026	AR-26-TH-012014-01-THPP1	AR-26-TH-012007-01-THPP1

Proteomics: ICS SOP 520-33 (2026-02), THPP1. The issued reports specifically mark this test as accredited. They assess D339H as non-compliant and D340H as compliant for the investigated parameters. “Negative” or “absent” means not detected under the applicable assay conditions, not a claim of absolute zero.

The DNA reports describe two classification models: at least one score was below its decision threshold in D339H; both were above threshold in D340H. Actual scores were not supplied. The reports state sensitivity 89.5% and specificity 99.6%; these are laboratory-reported figures, not independently verified GHIC estimates or probabilities that an individual sample is adulterated.

> **Assessment:** One concerning and one unremarkable case show compatible DNA and protein outcomes. Two samples cannot establish general sensitivity, specificity or market prevalence. Their agreement does not identify the adulterant fraction, the entry route or fraudulent intent.

4 · From a marker finding to a defensible explanation

Hypothesis	Evidence that helps distinguish it
Direct industrial addition or syrup carry-over	Specific peptide evidence; relevant syrup markers; feed/additive samples; procurement and blending records; linked lot findings.
Feeding-related entry	Feed composition and samples; feeding dates; separation of feed stores from harvested honey; harvesting and comb-management records.
Natural microbial source	Marker-specific ecological evidence; verified genuine regional honeys; organism and enzyme findings under realistic hive conditions.
Processing, storage or analytical artefact	Temperature/time and filtration records; recovery and digestion controls; repeat extraction; blanks, carry-over assessment and independent confirmation.

These explanations are not automatically equivalent. Their plausibility depends on the precise target and the complete evidence. Feed-derived sugar in the harvested product may itself raise a honey-definition issue; absence of post-harvest addition does not automatically establish compliance. [9, 12]

What strengthens a protein identification

Require documented selectivity against homologous proteins, suitable retention and fragment-ion criteria, controls for extraction and digestion, and safeguards against sample carry-over. Where technically feasible, GHIC proposes multiple discriminating peptides with appropriate reference materials or standards. This is a validation recommendation, not a claim that every current assay already uses an identical rule.

“Low” and “absent” bee-protein results need context: honey type, bee species within scope, processing, storage and analytical recovery. They can support a joint hypothesis but do not quantify dilution by themselves. Heat-related loss of activity and detectability of a peptide need not track each other in a fixed way.

DNA and proteins are genuinely different observations

Producer-organism DNA may be absent from a purified enzyme preparation even when its protein is detectable. Natural microbial DNA does not by itself demonstrate the corresponding enzyme preparation. The GHIC pair therefore shows molecular complementarity, not a requirement that every protein result must have a matching DNA hit.

5 · An operational response that preserves the evidence

1. Secure and delimit

Verify report authenticity, sample and lot identifiers, chain of custody and method scope. Preserve sufficient homogenised material for repeat work and a counter-sample. A justified temporary commercial hold can protect evidence and prevent further distribution while a qualified concern is clarified; it is not an automatic legal verdict.

2. Confirm the relevant analytical finding

Request the exact targets, identification criteria, applicable detection capability, controls and any reported qualifications. Repeat extraction where needed and obtain independent confirmation for a disputed or high-impact finding. Another negative sugar or DNA test cannot overrule a confirmed protein result unless it addresses the same hypothesis with adequate capability.

3. Investigate the route and the lot

Check feed practices, processing, additive purchases, blending and transport records. Compare retained producer, bulk and retail samples through documented lot relationships. Reconcile incoming honey, additions, outgoing product, stocks and losses in compatible mass units. A signed report or immutable hash protects the document history; it does not prove that the physical sample represented the whole shipment.

4. Classify and act on the complete evidence

Distinguish an unresolved suspicion, a substantiated compositional non-compliance, a food-safety concern and evidence of intentional deception. Where justified, provide the competent authority with the full dossier and preserve the operator’s opportunity to respond. The authority determines measures under the applicable framework. [9–11]

5. Make the wider method programme testable

Run blinded interlaboratory challenges using independently verified genuine honeys, authentic blends, enzyme-spiked materials, feeding controls and processing series. Predefine marker-specific false positives, false negatives, inconclusive results and robustness. Concentration-dependent detection of a protein is not the same as concentration-dependent detection of every syrup containing it. Publish disagreements and failure cases as well as successful identifications.

> For procurement: buy a specified analytical question, a documented method and an interpretable report. Do not buy the promise that one panel can certify every dimension of authenticity.

6 · Public policy, legal framework and implementation

Act within a defined evidential and legal scope

The EU Honey Directive defines honey and prohibits additions other than honey. Meeting a routine compositional value does not override that definition. Article 4 provides for implementing acts on methods by 14 June 2028; this is not a suspension of current controls. Method selection must satisfy the applicable legal hierarchy and fitness requirements. [9, 10]

Under Regulation (EU) 2017/625, Articles 34–35 govern methods and the right to a second expert opinion; Article 37 addresses official laboratories. Articles 137–138 distinguish investigation of suspected non-compliance from measures following established non-compliance. Measures must be justified for the case; neither a private model flag nor an accreditation logo automatically decides the legal outcome. [10]

Operators are responsible for compliance and traceability. Food-safety withdrawal and notification duties under Article 19 of Regulation (EC) 178/2002 must be assessed against its conditions; an authenticity issue is not automatically a demonstrated safety hazard. Deliberate fraud requires additional evidence of deception and responsibility. [11]

A concrete GHIC implementation proposal

- First 30 days: laboratories, authorities, producers and buyers agree the questions, sample plan, data fields, confidentiality rules and independence safeguards.
- Days 31–60: controlled split samples, reference materials and blinded challenges establish an auditable pilot dataset; unresolved identity or quality failures remain visible.
- Days 61–90: review concordant and discordant findings, publish a preliminary methods report and define the larger interlaboratory validation. A 90-day pilot does not equal completed validation.

Public-interest safeguards

Separate laboratory findings from commercial interests and case advocacy. Declare funding and conflicts, preserve counter-samples and correction records, and offer controlled scientific access to necessary data while protecting legitimate trade secrets. Report results proportionately and avoid unsupported public attribution of fraud.

Success means faster resolution of credible concerns, fewer unsupported accusations, better protection for honest beekeepers and more defensible purchasing and enforcement decisions. All origins and business models must face the same evidential standard.

Sources and editorial basis

Evidence cut-off: 17 September 2026. Targeted review of primary research, EU legislation and the supplied laboratory records; not a systematic review. [1, 3, 5] were assessed using indexed abstracts and bibliographic records; [2] is a scientific comment, not an experimental validation. [8] is a provider publication. Source titles remain in their original language; links are clickable.

[1] Paluoja et al. (2025). Honey bulk DNA metagenomic analysis to identify honey biological composition and monitor honey bee pathogens. npj Science of Food 9, 91. DOI ↗

[2] Pellegrin, Linsinger & Alvarellos (2026). Of bees and buzz: towards validated NGS-based methods in honey authentication. npj Science of Food 10, 129. DOI ↗

[3] Erban et al. (2021). A single honey proteome dataset for identifying adulteration by foreign amylases and mining various protein markers natural to honey. Journal of Proteomics 239, 104157. DOI ↗

[4] Takahashi et al. (2023). Investigation of Foreign Amylase Adulteration in Honey Distributed in Japan by Rapid and Improved Native PAGE Activity Staining Method. Journal of Applied Glycoscience 70, 67–73. DOI ↗

[5] Erban et al. (2026). Mining foreign proteins in honey by shotgun label-free proteomics: Implications for quality and authenticity. Journal of Proteomics, 105657. DOI ↗

[6] Wang et al. (2025). Bacillus subtilis Contributes to Amylase Production in the Honey Sac of Apis mellifera. Insects 16, 221. DOI ↗

[7] GHIC (24.07.2026). GHIC–Celvia Orthogonal Concordance Report, ET/EN. Supplied case analysis and embedded Celvia / Eurofins reports; D339H and D340H.

[8] Eurofins / Witt & Kunert. Proteomics: a new method for the detection of honey adulteration. White paper, update 21.02.2026. Source ↗

[9] European Union. Directive 2001/110/EC relating to honey, consolidated 14.06.2026; Article 4 and Annexes I–II. Source ↗

[10] European Union. Regulation (EU) 2017/625 on official controls; Articles 34–35, 37, 137–138. Source ↗

[11] European Union. Regulation (EC) 178/2002; Articles 17–19. Source ↗

[12] European Commission. EU coordinated action “From the Hives” (2021–2022): results and questions and answers. Source ↗

[13] ISO. ISO/IEC 17025:2017: General requirements for the competence of testing and calibration laboratories. Public standard overview. Source ↗

> Publisher: Global Honey Integrity Council (GHIC). Newly developed GHIC positions in five equivalent language editions. The earlier EPBA DNA paper is historical background, not a current EPBA endorsement. The case report [7] is an unpublished, two-sample GHIC analysis; its findings are paraphrased here without reproducing confidential report images or personal data. Recommendations are GHIC proposals, not pre-existing legal or laboratory requirements.