Table S1. Relationship between pre-course diagnostic questions and official high school curriculum content. This table maps the 12 diagnostic questions to the official Spanish high school curriculum. The content was verified against the LOMCE (Organic Law for the Improvement of Educational Quality, established by Royal Decree 1105/2014) and the current LOMLOE (Organic Law for the Modification of the LOE, established by Royal Decrees 217/2022 and 243/2022).

Nº	Question	Semester	Official Curriculum Content	Course and Subject	Specific Unit/Block
1	Non-polar substances are:	1	Molecular polarity, chemical bonds, hydrophobic interactions	4th Compulsory Secondary Education - Biology and Geology	Living matter: bioelements and biomolecules
2	The peptide bond is:	1	Peptide bond, primary structure of proteins	1st Upper Secondary Education - Biology and Geology	Proteins: structure and functions
3	In a hyperbolic enzyme kinetic, half of the maximum velocity is reached when:	1	Enzyme kinetics: Km and Vmax, Michaelis-Menten model	2nd Upper Secondary Education - Biology	Enzymes: kinetics, mechanisms, and regulation
4	A competitive inhibitor of an enzymatic reaction:	1	Enzyme inhibition: competitive, non-competitive	2nd Upper Secondary Education - Biology	Enzymes: regulation of enzymatic activity
5	Which of the following statements about nucleotides is true?	1	Nucleotides as structural and energetic units	1st Upper Secondary Education - Biology and Geology	Nucleic acids: structure and functions
6	Protein synthesis...	1	Transcription, translation, messenger RNA and ribosomes	1st and 2nd Upper Secondary Education - Biology	Gene expression: protein synthesis
7	Energy charge is defined as (...) what will it determine?	2	Cellular energy charge, metabolic regulation	2nd Upper Secondary Education - Biology	Metabolism: energetic control and regulation
8	What is the name of the transformation of pyruvate to malate by the malic enzyme?	2	Anaplerotic reactions, intermediate metabolism	2nd Upper Secondary Education - Biology	Metabolism: catabolic pathways and accessory functions
9	What are the implications of cytosolic glucose phosphorylation?	2	Glycolysis: first steps, regulation by phosphorylation	2nd Upper Secondary Education - Biology	Glycolysis and enzymatic regulation
10	How do animal cells obtain the necessary NADPH for biosynthesis?	2	Pentose phosphate pathway, NADPH as a biosynthetic reducer	2nd Upper Secondary Education - Biology	Anabolic pathways: pentose phosphates and functions of NADPH
11	In the β-oxidation of fatty acids,	2	Lipid degradation, energy production by β-oxidation	2nd Upper Secondary Education - Biology	Lipid metabolism: β-oxidation
12	Which hormone activates glycogen synthesis?	2	Hormonal regulation of carbohydrate metabolism (insulin)	2nd Upper Secondary Education - Biology	Hormonal regulation: insulin, glucagon, adrenaline