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Durch die Verwendung einer FKS-Ersatzlösung für einen Routinetest könnte darüber hinaus die Nachfrage nach fötalem Kälberserum gesenkt und damit der tierschutzwidrigen Gewinnung dieses Produktes vorgebeugt werden. Bei der Verwendung synthetischer Medien wäre auch die Verwendung von Plazenta-Extrakten ausgeschlossen, deren Herstellung allerdings weniger tierschutzrelevant ist als die Gewinnung von FKS. Plazenten fallen bei jeder Kälbergeburt als Abfallprodukt an und werden auch für die Weiterverarbeitung in kosmetischen Produkten gesammelt. Durch den Ersatz des fötalen Kälberserums könnte damit sowohl aus wissenschaftlicher, als auch aus Sicht des Tierschutzes die Akzeptanz des Zytotoxizitätstests mit Fischzellen als Alternative zum Fischtest insgesamt verbessert werden.

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Endomysium Antibodies Detection with Umbilical Cord Sections

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Zusammenfassung: Nachweis von Endomysium-Antikörpern mit Nabelschnur-Schnitten.
Der Endomysium-Antikörpernachweis (EmA) gilt als zuverlässigster Test zur Diagnose der Zöliakie. Üblicherweise wird ein indirekter Fluoreszenztest an Schnitten von Affen-Speiseröhren durchgeführt. Beim Vergleich eines Anti-Gliadin (AGA) ELISA´s an Affen-Speiseröhren- und Nabelschnur-Schnitten zeigte der Test bei beiden Geweben die gleiche Sensibilität, beim Gebrauch menschlicher Nabelschnüre jedoch eine höhere Spezifität. Der Ersatz der ethisch bedenklichen Verwendung von Affen-Gewebe durch die Verwendung von Nabelschnüren vermindert zudem die Testkosten.

Summary
Anti-Endomysium antibodies (EmA) detection is the most reliable test for the diagnosis of coeliac disease. Usually monkey distal oesophagus sections are used. Tests with ELISA anti-gliadin (AGA) antibodies show that umbilical cord EmA tests can effectively substitute monkey oesophagus EmA tests.

Keywords: Coeliac disease, endomysium antibodies, gliadin antibodies, monkey oesophagus, umbilical cord

1 Introduction

In the past few years, the Anti-Endomysium antibodies detection (EmA) has been revealed as the most reliable test for the diagnosis of coeliac disease. Current tests normally used in diagnostic laboratories utilize an Indirect Immunofluorescence (IFI) protocol with monkeys distal oesophagus sections. Some authors (Landinser et al., 1994) have been researching the possibility to substitute monkey tissues with umbilical cord tissues, with the purpose to overcome ethical facets linked to the utilization of dead animal tissues and to allow a significant decrease of the price of EmA tests, since umbilical cord is a tissue that is normally discarted, does not have endogenous immunoglobulins and is easy to find. A study has been pursued on a pediatric population encompassing 105

patients (80 coeliacs and 25 control subjects), in order to verify the reliability of umibilical cord in the process of EmA detection. Sera belonging to all patients were tested with ELISA anti-gliadin (AGA) IgA and IgG antibody tests and two different IFI methods for the detection of EmA, utilizing monkey oesophagus and umbilical cord sections respectively. Final results underline the overlapping sensitivity of the two IFI methods similar to ELISAs. Above all, the IFI test with umbilical cord section showed a greater specificity compared to all tests performed.

2 Methods

The present study involved 105 patients aged between 1-15 years, 80 of which with coeliac disease confirmed by intestinal biopsy, and 25 controls. All patients were submitted to a peripheral venous blood drawing. Blood collected was centrifuged and the obtained sera were stored at -20° C until dosage. Such sera have been defrozen and tested at the same time for IgA, IgG AGA and EmA with monkey oesophagus and umbilical cord sections. Dosages were performed by two different operators, none of them knowing the biopsy results.

2.1 IgA and IgG AGA Dosage

An ELISA kit expressing results in mg/l has been used to assay IgA and IgG AGA. Results were quantified by a six point scale standard curve obtained through the dilution of a standard having a 6.4 mg/l concentration. Cut-offs were set as 0.2 mg/l for IgA and 0.6 mg/l for IgG and patients sera were diluted 1:200 for both ELISA tests.

2.2 EmA Dosage

Two kits with 60 sections of monkey oesophagus and umbilical cord each were used for EmA dosage. Both kits contained all necessary material to perform the test, including positive and negative controls. Patients sera were diluted 1-5 for both EmA tests. After having added serum onto sections and having performed three washing steps, 40 mL of prediluted anti-IgA FITC were added. After further washes, mounting medium was added. Then, slides were ready to be viewed with a fluorescence microscope at 250-400x. In the monkey oesophagus sections, positivity was confirmed by the presence of fluorescence in the endomysial connective tissue of muscularis mucosae and external muscularis tunica. In the umbilical cord sections, positivity was confirmed by the presence of fluorescence in the matrix proteins surrounding smooth muscle fibres in the whole of the umbilical vein and arteries (figure 1).

3 Results

Obtained results are reported in table 1.
A 100 % concordance has been obtained in the diagnosis of the 80 coeliac patients (80/80) using the AGA IgG kit, while a 96.2 % concordance is shown using the AGA IgA kit (77/80). A 97.5 % concordance has been obtained in the diagnosis of the 80 coeliac patients (78/80) using either umbilical cord or monkey oesophagus in the EmA IFI kits.

A 36 % concordance has been obtained in the diagnosis of the 25 control patients (9/25) using the AGA IgG kit, while a 68 % concordance is showed using the AGA IgA kit (17/25). A 96 % concordance has been obtained in the diagnosis of the 25 control patients (24/25) using monkey oesophagus and 100 % (25/25) using umbilical cord.

4 Discussion

In subjects showing a selective intolerance to gluten an increase of anti-gliadin and anti-endomysium in serum has been noticed. It is fundamental that diagnostic tests furnish reliable and timely results, due to the importance of the coeliac disease. Results obtained clearly point out how AGA IgA and IgG ELISA tests are extremely useful in the detection of coeliac patients, notwithstanding they show some weakness in specificity (presence false positives). However, such tests can be useful for the screening of population because of their low cost. On the other hand, EmA tests can be utilized as confirmatory tests due to their high specificity. Our results confirm these data: ELISA AGA tests showed high sensitivity while EmA tests showed high specificity. In addition, the comparison between the two EmA tests revealed that both of them have the same sensitivity (97.5 %), even though the umbilical cord EmA test seems to hold a better specificity (100 %). Our data show that umbilical cord EmA tests can effectively substitute monkey oesophagus EmA tests contributing to solving problems related to high cost kits and ethical problems connected with the endangered species.

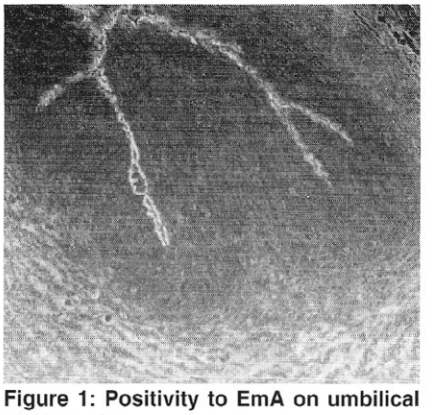

Figure 1: Positivity to EmA on umbilical cord section.

	Patients	AGA IgA	AGA IgG	EmA oesophagus	EmA umbilical cord
Celiacs	80	77 (96,2%)	80 (100%)	78 (97,5%)	78 (97,5%)
Controls	25	17 (68%)	9 (36%)	24 (96%)	25 100%)

Table 1: Concordance in the diagnosis of coeliac patients with different test-kits

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