Comparative analysis of anti-measles antibody detection of laboratory techniques:

Immunoserological assay development for the assessment of long-term measles/MMR vaccine efficacy with practical and theoretical benefit

Ph.D Thesis

Katalin Böröcz

Supervisor:
Prof. Péter Németh M.D., Ph.D.

Programme leader:
Prof. Tímea Berki M.D., Ph.D., Ds.C.

Leader of the Doctoral School:
Prof. Dóra Reglődi M.D., Ph.D., Ds.C.

Department of Immunology and Biotechnology
Medical School
University of Pécs, Hungary

P é c s

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1 SYNTHESIS

Infectious immunity has an outstanding importance today. Vaccination remains one of the safest and most effective interventions available in public health for the primary prevention of infectious diseases, resulting in both direct and indirect (herd immunity) immunity in individuals vaccinated (1–3). Even though in Europe a safe and effective two-dose measles/MMR vaccination schedule has been made available since the 1960s, the maintenance of high vaccine coverage is still difficult (4–7). Despite the fact that in Hungary the measles (nowadays MMR) vaccine is mandatory since 1969, and consequently the vaccine coverage is estimated at 99% (WHO), vaccination-group specific immunization gaps may exist (8–15). Suboptimal vaccine effectiveness in certain vaccination -or age- groups has a negative impact also on overall vaccination coverage. The small-scale 'measles outbreak of Makó and Szeged' (2017) confirmed that certain measles vaccines - applied during the early phases of the Hungarian vaccination history –, failed to elicit the desired immunological response. The resulting immunization gap(s) raise the concern of potential further outbreaks (10,15). Screening for immunity and effectiveness of vaccination against infectious diseases has increasing importance in the design of preventive public health strategies, especially today, when prompt testing is also emphasized by the ongoing COVID-19 pandemic. Measles has been already an issue worldwide, yet being aggravated by disrupted immunization protocols due to the COVID-19 burden (4–6,16). Immunity gaps arising from suspended immunization activities are an ominous precursor to a measles resurgence (4). Measles is extremely communicable with the basic reproduction number (R_0) estimated at 12-18 (compared to 2.9-3.4 of COVID-19, for example). Accordingly, we have developed a robust, time-saving, cost-effective and standardized 'triple' immunoserological assay for simultaneous detection of anti-measles, -mumps,

and -rubella IgG antibodies in human sera. Since our test has been optimized for the screening of suboptimal antibody titers, it is able to operate reliably in the low measurement range, therefore can be readily used to delineate susceptible individuals and gaps of immunological protection.

In addition to the practical benefit, the present study also has a theoretical importance; comparison of adaptive antibody levels with natural (auto)antibody (nAAb) levels that for a long time have been thought to lack the capacity of dynamic adaptation. We intended to find quantitative data for a new approach in vaccination; natural autoantibodies may play a role in efficient vaccination (95), and the unforeseen benefit of immunization may lie in the potential enhancement of natural antibody pool (17). For this reason, we compared vaccine- (or pathogen-) induced antibody levels (elicited by the historical measles/MMR vaccine, with >99% of current vaccination coverage, strengthened by epidemics in the early decades of vaccination) with nAAbs (anti-citrate synthase [anti-CS]), anti-DNA topoisomerase I F4 fragment [anti-F4]) and double stranded DNA [anti-dsDNA], of patients with systemic autoimmune diseases (SAIDs) as SLE, RA and SSc. Simultaneously, we also investigated the potentially impaired memory B cell functions in autoimmune diseases by comparing the population-level anti-measles IgG antibody titers to that of patients with SAIDs.

2 AIMS

I. Assay development: optimization of an efficient tool for the screening of suboptimal measles/MMR humoral antibody levels

II. Sero-epidemiology: large-scale screening of MMR antibodies at population level

III. Comparative study for the assessment of potentially impaired immune-regulatory functions in systemic autoimmune diseases: analysis of the potential link between naturally occurring (auto)antibodies, and vaccine –or infection– induced antibodies

3 MATERIALS AND METHODS

3.1 Assay development

3.1.1 Establishment of anti-measles, -mumps and -rubella IgG Indirect ELISA operational protocol (OP)

3.1.2 Plate and coating buffer selection

3.1.3 Testing for potential coating-related interferences

3.1.4 Blocking and diluent optimization

3.1.5 Calibration curve and serum antibody quantification

3.1.6 Optimal dilution of samples

3.1.7 Determination of cut-off values

3.1.8 Instrumentation platform

3.1.9 Software, statistical data evaluation

3.1.10 Assay cost, and execution time

3.2 Sero-epidemiology

3.2.1 Samples

3.2.2 Categorization

3.2.3 Sero-epidemiological evaluation

3.3 A potential link between natural (auto)antibodies and vaccine –or infection– induced antibodies

3.3.1 Samples

3.3.2 Comparative ELISA measurements

3.3.3 Statistical analysis

4 RESULTS I – ASSAY DEVELOPMENT

4.1 ELISA plate surface optimization

The ability of the surface to interact with proteins and other biomolecules is essential, however, non-specific binding (NSB) of other proteins or biomolecules to unoccupied spaces on the surface during subsequent steps of the assay can be detrimental to the specificity and sensitivity of the assay results. Therefore, the here presented results are to be interpreted in the context blocking ad diluent optimization trials. Of the tried solid surfaces, Nunc Maxisorp high-binding plate was selected, because of negligible backgrounds, well-fitting (R^2) and adequately high standard curves, as well as successful parallelism test (represented as a supplementary figure of our paper (15).

4.2 Antigen coating purity testing

The 'three-in-one' MMR ELISA - that is in fact a compacted total virus antigen repertoire based anti-measles, -mumps and –rubella IgG indirect ELISA - was considered as 'target', while recombinant, nucleocapsid based ELISAs (of the same viruses) were considered as 'control' tests. Assays were contrasted to check whether the cell culture derived, entire virus based coatings contain off-target molecules that may lead to unwanted interference and consequent false-positive results. For this purpose, Bland-Altman plots have been used; ratios of the results from the two techniques ('target' versus 'control' assay) were plotted against the averages. We obtained data points that fell within the range ± 1.96 SD (confidence interval 95%), with no observable trends, suggesting that the two methods are in agreement, thus demonstrating the adequate purity of the entire virus based coating system used in the 'target' assay (15).

4.3 Blocking and diluent optimization

As described in our article (14), blocking solutions (protein-containing and protein-free) were tested on plates that had not been coated with antigen; only blocking solutions only were applied to 'coat' plates (overnight at 4-6 °C), than

analytes were tested as per OP. We demonstrated that using Block ACE and bovine skin gelatin, the absorbance values reflect the increasing concentration of the standard, which suggests non-specific interactions. Such non-specific reactions were not observed in the case of SynBlock and our polyvinyl alcohol (PVA) based synthetic blocking solution, used as a cost-effective alternative of SynBlock. Therefore, for our subsequent experiments we used the PVA based synthetic blocking solution (14).

4.4 Background reduction

In a serological assay that is targeted for the detection of low, or suboptimal results, it is crucial to get rid of as many interference (and subsequent false-positivity) inducing factors, as possible. In the light of this principle we used the so called IgM reducing buffer (IgM RB) that resulted efficient not only in the elimination of IgM, but also in the reduction of unwanted uncategorized bindings.

4.5 Cut-off determination and immune-assay precision

To test assay precision, we compared our self-developed test to well-established, commercially available kits. Cohen's Kappa 'plate-to-plate κ statistics' gave 'substantial' to 'near perfect' agreement; $0.64 \leq \kappa \leq 0.92$. The AUROC analysis - used to set the cut-off values – gave results ≥0.92, for all three antigens. Based on the AUROC analysis, with the help of Youden's equation, the following sensitivity-specificity pairs were selected 0.985 – 0.975, 0.935 – 0.911, 0.989 – 0.946 for measles, mumps and rubella, respectively (15).

According to the 'empirical approach' -already detailed in our earlier paper (14)-, cut-off values were also set for all antigen types (measles, mumps, rubella) based on mean observed OD values belonging to diagnostically seronegative samples (3x15 samples, OD negative sample ≤ 0.28, 0.37, 0.34 for measles, mumps, and rubella, respectively). Cut-off values calculated based on empirical results were concordant with the statistically computed values.

4.6 Optimal dilution of samples

The optimal dilution of samples was found to be 200-fold (0.005 relative concentration), performed in two steps, combined with IgM reducing assay diluent (14). This method yielded an acceptable signal and reproducible difference between positive and negative samples, with minimal use of stock solutions (14,15).

4.7 Parallelism testing

The measured absorbance (OD) of samples plotted versus relative concentration resulted in saturation curves similar to the calibration curve, thus 4-parameter logistic curves were fitted. Dilution curves of two, typical low-titer samples and two high-titer samples were linearized by taking the common logarithm of the dilution and the calculated concentration. Linear fit was performed with slope -1 (determined from previous assays) for each data set. R-square values were close to 1 (0.91-0.99), which suggested that the binding characteristic of the analyte (serum antibodies) to the antigen were co-measurable to the standard. A better linear fit was observed for higher titer samples, because of the better signal-to-noise ratio of the spectrophotometric method in the measured OD range (14).

4.8 Agreement between tests

In addition to the already detailed comparisons (also used for coating-purity testing) (14,15), comparability to commercially available kits was also investigated. Plate-to-plate Cohen's Kappa 'κ' statistics gave 'substantial' to 'near perfect' agreement; $0.64 \leq \kappa \leq 0.92$ (15).

4.9 Immuno- assay characteristics: cost, ease, time-saving

An important feature of our 'three-in-one' MMR ELISA assay is affordability; it costs only a fraction of the price of commercially available assays (15). Another important feature is the reduced assay duration time; compared to the ~ 1.5 / 2.5 hours of timeframe of commercially available tests our test can be performed within 1 hour.

5 RESULTS II – SERO-EPIDEMIOLOGY

5.1 Vaccination history timeline

Changes, and historical data regarding epidemics in the Hungarian measles/MMR vaccination schedule (8,18,19) were plotted on a timeline, in order to evaluate sero-epidemiological data accordingly. Data were collected and analyzed, thus changes, insufficiencies and the possible underlying causes of the epidemics throughout the Hungarian measles/MMR vaccination history were mapped. Using this representative timeline (8,11), it has been evidently demonstrated that high age specific attack rates characterized the major epidemics (1980-81 and 1988-89), along with 93% - 99% of vaccine coverage.

5.2 Determination of age-groups with highest frequencies of seronegativity

Considering the relative herd immunity threshold (HIT) values (HIT Measles = 92–95%, HIT Mumps = 75–86%, HIT Rubella = 83–86), anti- measles IgG seropositvity ratios proved to be inadequate in certain clusters of the population. The lowest seropositvity ratios (N total measles = 3523 serum samples) were detected in clusters ‘Vaccinated between 1978 and 1987’ (~80% of seropositvity) and ‘Vaccinated between 1969-77’ (~90% of seropositvity). Analyzing the vaccination period-specific confidence intervals of seronegativity; the group ‘Vaccinated between 1978 and 1987’ showed significant differences from the flanking age-groups; ‘Vaccinated between 1969-1977 and 1988-1990’ ($p = 0.00004$ and $p = 0.0015$, respectively). In the case of rubella (N = 1736 serum samples), the least protected groups were vaccinated during 1969-1977 (~85% of seropositvity) and 1988-1990 (~85% of seropositvity). Significant differences were observed between the group born before 1969 (not vaccinated) and vaccinated during 1969-1977 ($p = 0.00008$), and between groups 1988-1990 and 1991-1995 ($p = 0.009$). In case of measles, mumps and rubella cumulative results, the seropositvity ratios were 89.97%, 91.60% and 92.58%, respectively. In practical terms, it means that in case of measles, due to cluster-specific inadequacy of IgG levels, also the overall seropositivity ratio (measles = 89.97%) failed to reach the

herd immunity threshold (HIT Measles = 92–95%). Considering mumps and rubella, herd immunity – in terms of humoral antibodies –was reached (7).

6 RESULTS III - A POTENTIAL LINK BETWEEN NATURAL (AUTO)ANTIBODIES AND VACCINE– OR (INFECTION–) INDUCED ANTIBODIES

6.1 Vaccine– (or infection–) induced anti-measles IgG seropositvity ratios in systemic autoimmune diseases

Anti-measles IgG levels in samples obtained from all patients of the sample group *'systemic autoimmune diseases'* (SAD) (total n=374) were measured, then seronegativity ratios were also evaluated. As it is already described in epidemiological literature, and in accordance with our previously published findings (8,11,14,15,18); measles seronegativity ratios showed significant correlation with age ($p<0.001$ correlation coefficient; 0.323), which is a general phenomenon, not linked to autoimmunity. Considering the seronegativity ratios; between the earlier detailed population-level 'overall' anti-measles (IgG) result (10.03% of seronegativity) and the current autoimmune disease focused sample multitude (8.82% of seronegativity) we found no remarkable difference.

6.2 Comparison of natural autoantibody and vaccine– (or infection–) induced antibody levels

Based on previous findings nAAbs (IgG/M) against the mitochondrial inner m)embrane enzyme citrate synthase and topo I F4 could be detected in sera of healthy individuals and patients with SSc, SLE and in other autoimmune rheumatic diseases (20). When analyzing the undivided totality of 'systemic autoimmune diseases' sample group (total n=374), the same trend was observed as in case of the three accentuated disease groups (n SSc=157, n SLE = 92, n RA = 73, n other = 52). Considering all the *'systemic autoimmune diseases'* sample group (without further subdivisions); significantly higher anti-CS IgG titers were detected in the anti-measles IgG seropositive patient group ($p = 0.011$) (21).

Analyzing the association between measles vaccine or virus- -induced (anti-measles IgG) and natural (anti-CS IgG) antibody titers in the individual

autoimmune diseases (RA, SLE and SSc), the same trend described above was observable; in all three groups the anti-measles IgG seropositive samples showed significantly higher anti-CS IgG titers ($p = 0.035$, $p = 0.041$ and $p = 0.039$ for RA, SLE and SSc, respectively). Similar, but statistically not significant trend was observed in case of anti-F4 IgG nAAbs (21).

6.3 Connection between IgG isotype natural autoantibodies and anti-dsDNA IgG levels in SLE patients

In order to investigate the association between autoimmune disease-specific pathological autoantibodies and IgG isotype nAAbs, anti-dsDNA IgG measurement was chosen, as anti-dsDNA IgG is a highly specific disease marker in SLE. In those SLE patient samples that proved to be positive for the disease specific marker; anti-dsDNA IgG, significantly higher levels of anti-F4 IgG ($p = 0.001$) and anti-CS IgG ($p < 0.001$) nAAbs were found. Correlations also proved to be significant between anti-dsDNA IgG and the IgG isotype nAAbs (p / correlation coefficient: 0.006 / 0.321 and 0.000 / 0.510 for anti-F4 IgG and anti-CS IgG, respectively) (21).

6.4 Anti-dsDNA IgM and natural IgM autoantibody levels showed association in SLE

Previous reports proposed that anti-dsDNA IgM antibodies may play a protective role in lupus nephritis (22–24). Herein we compared anti-dsDNA IgM levels with nAAb titers in SLE patients. It was found that in anti-dsDNA IgM positive SLE patient samples, significantly higher levels of anti-F4 IgM and anti-CS IgM nAAbs were also detectable ($p = 0.002$ and 0.016, respectively). Anti-dsDNA IgM titers and nAAb levels also showed significant correlation (p/correlation coefficient: 0.002/0.344 and 0.018/0.252 for anti-F4 IgM and anti-CS IgM, respectively) (21).

7 CONCLUSION

We have developed a high throughput, time-saving, cost-effective immunoserological assay that relies on international standards, for simultaneous detection of anti-measles, -mumps, and -rubella IgG antibodies in human sera. This test – to the difference of many commercially available immunoserological kits – has been optimized with the pronounced purpose of maximal background and interference removal, in order to enable reliable detection of suboptimal antibody titers. Our 'triple' or 'three-in-one' assay uses the same reagent load with uniform, short incubation times and equally pre-treated samples, enabling the three-parametric screening of 24 samples per plate within one hour, manually, or in an automated platform. In high throughput automated settings, separate testing of the three antigen types is also feasible, thus allowing the measurement of 80 samples per run.

We conclude that the importance of sero-epidemiological surveys is confirmed by recent outbreaks of measles, mumps, and rubella infections in several countries (25–33). Vaccine effectiveness monitoring is especially important nowadays, when the already dubious immunization practices in some of our neighboring countries have been aggravated by the detrimental effect of the COVID-19 pandemic. The subsequent suspension of measles vaccination campaigns may facilitate the occurrence of smaller importation-related MeV outbreaks in susceptible cohorts (4–6,16).

We analyzed serum samples (N total measles = 3919 measles, N mumps = 2132 mumps, and N rubella = 2132). Considering the HIT values, suboptimal anti-measles seropositivity ratios were detected in certain clusters of the early vaccination era (≈80 % of sufficient anti-measles IgG antibody titers among individuals vaccinated between '1978 and 1987'). This finding – in accordance with recent publication (10) and previous literature data (11) - suggests the

existence of age-specific immunization gaps in the Hungarian population. For mumps and rubella, our data shows satisfactory immunity levels. We would like to emphasize that today in our country, the MMR vaccination coverage is ideal, due to the mandatory administration of safe and modern trivalent vaccines. The revealed gaps at population-level humoral immunity (IgG) are specifically linked to the early vaccination period, and are not a general phenomenon relative to current immunization practices (7,14,15).

Regarding nAAbs, our results – supported by literature data (38-40) -, suggest that the natural antibody pool, which was thought to be constant over time, is in fact capable of a certain degree of dynamic adaptation, which also implies the recognition of evolutionarily fixed epitopes not only of self, but also of foreign antigens (36,37). Today it is supposed that natural antibody repertoire is inherently linked to the host biome (17,38). It may explain, how vaccination - one of the main pillars of modern medicine-, induces not only the formation of memory B-cells and antibodies that confer immunity to disease causing pathogens, but also has an unintended impact on the natural antibody repertoire (17,39). The unforeseen benefit of vaccination; the enhancement of natural antibody networks, has been also reported (17). Our measurements demonstrate that there is a significant positive connection between IgG isotypes of vaccine- (or pathogen-) induced antibody levels and natural antibody levels, hence may serve as a confirmation of this theorem.

Considering nAAbs, still many paradox findings are described by literature. These may be, at least, partly explained by the many diverse approaches, each focusing on different research targets. The long-term vaccine effectiveness monitoring - using historical, well-established vaccines, as objects - may serve as a tool to resolve these contradictions. By pragmatic means; using immunoserology -based comparisons of vaccine- (or pathogen-) induced ‘adaptive’ IgG levels to nAAb titers, we can answer yet debated questions regarding the natural human IgG antibody repertoire (e.g. life-long stability of reactivity towards self-antigens in

contrast with age-dependent diversification of reactivity against foreign antigens (40)).

The dogma that high-affinity IgG response is the major goal of immunization and low-affinity Abs should be avoided, has positively contributed to the lack of information regarding the role of nAbs in vaccination (37). However, it has been recently proposed that nAbs may serve as potential screening targets to predict the strength of antigen-induced immune response (41).

8 SUMMARY OF NOVEL RESULTS

1. We have developed a high throughput, time-saving, cost-effective and standardized ‘triple’ immunoserological assay for simultaneous detection of anti-measles, -mumps, and -rubella IgG antibodies in human sera. Since our test has been optimized for the screening of suboptimal antibody titers, it can be readily used to delineate susceptible individuals and potential gaps of immunological protection.
2. Our ‘triple’ or ‘three-in-one’ immune-assay uses the same reagent load with uniform, short incubation times and equally pre-treated samples, enabling the three-parametric screening of 24 samples per plate within one hour, manually, or in an automated platform.
3. Using our self-developed assay(s) (validated using well-established, commercially available kits), we have specified the age-range of those potentially susceptible cohorts, who have received the measles vaccine during the early times of vaccination history (between 1969-77 and between 1979-87) in the Hungarian population.
4. This background-noise reduced and signal-to-noise ratio optimized ELISA method that we have developed, has also gained a potential secondary benefit in the meantime; it could be used as a base for a similar immuno-assay development in the respect of COVID-19 vaccine assessment, and in the screening for suboptimal antibody titers and non-responder vaccinees.
5. We have demonstrated - using the measles vaccine – induced immunological memory as a ‘tool’- that there is a significant positive connection between IgG isotypes of vaccine- (or pathogen-) induced antibody levels and natural antibody levels. It may give a functional evidence to the supposition that attributes adaptive capacity to natural antibodies of IgG isotype.

6. We found a quantifiable, numerical (measurable antibody levels) proof demonstrating that the natural antibody pool, which was thought to be constant over time, is in fact capable of a certain degree of dynamic adaptation
7. Our assay – applied for post-vaccination follow-up studies - can be used as a functional test of potentially impaired memory B cell functions in patients with autoimmune diseases
8. We found an easily feasible, cheap immunoserological method to answer yet debated questions regarding the natural human IgG antibody repertoire (e.g. life-long stability of reactivity towards self-antigens in contrast) with age-dependent diversification of reactivity against foreign antigens

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9 LIST OF RELATED PUBLICATIONS

Böröcz K, Csizmadia Z, Markovics Á, Mészáros V, Farkas K, Telek V, Varga V, Maloba G O, Bodó K, Najbauer J, Berki T, Németh P. Development of a robust and standardized immunoserological assay for detection of anti-measles IgG antibodies in human sera. J Immunol Methods. 2019 Jan 1; 464:1–8.

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10 LIST OF OTHER PUBLICATIONS

Bodo, K; Boros, A ; Rumpler, E ; Molnar, L ; Borocz, K ; Nemeth, P ; Engelmann, P. Identification of novel lumbricin homologues in Eisenia andrei earthworms. DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 90 pp. 41-46. , 6 p. (2019)

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Berki, T; Hayden, Z ; Böröcz, K ; Csizmadia, Z ; Kellermayer, Z ; Balogh, P. Immunmediált kórképek diagnosztikája : laboratóriumi szakemberek és a klinikusok együttműködése. NEUROLÓGIAI PRAXIS 1 : 4 pp. 14-15. , 2 p. (2018)

Borocz, K; Hayden, Z; Meszaros, V ; Csizmadia, Z ; Farkas, K ; Kellermayer, Z ; Balogh, P ; Nagy, F ; Berki, T. Az autoimmun encephalitisek laboratóriumi vizsgálati lehetőségei. ORVOSI HETILAP 159 : 3 pp. 107-112. , 6 p. (2018)

11 SCIENTIFIC ACTIVITIES

OCCASION	DATE	PLACE	POSTER/ PRESENTATION	TITLE
46th Membrane-Transport Conference	17-20 May, 2016	Sümeg, Hungary	Poster and presentation	**Diagnostic value of anti-receptor autoantibodies in immune-mediated neurological diseases** *Böröcz K, Mészáros V, Kellermayer Z, Berki T*
45th Annual Roving Conference of the Hungarian Immunological Society	19-21 October, 2016	Velence, Hungary	Poster	**Large-scale immunoserological efficacy study of measles vaccination** *Böröcz K, Mészáros V, Farkas K, Csizmadia Z, Katz Z, Németh P*
Center for Immunological Excellence - Seminars	11 May, 2017	University of Pécs, Pécs, Hungary	Presentation	**Obstacles and successes during the development of an automated ELISA test, used for the detection of anti-measles antibody detection** *Böröcz K – Németh P*
47th Membrane-Transport Conference	16-19 May, 2017	Sümeg, Hungary	Poster	**Modeling the conformation sensitivity of antigen-antibody binding on artificial surfaces** *Böröcz K, Csizmadia Z, Mészáros V, Berki T, Markovics Á, Németh P*
46th Annual Roving Conference of the Hungarian Immunological Society	18-20 October, 2017	Velence, Hungary	Presentation	**Measles vaccine efficacy study- identifying potentially susceptible cohorts in Hungary** *Böröcz K, Ouma M G, Csizmadia Z, Farkas K, Németh P*
5th Autoimmunity Seminar – Shedding a new light on autoimmunity	13-15 November, 2017	Barcelona, Spain	Presentation	**Comparison of autoimmune routine diagnostic laboratory elisas with their corresponding chemiluminescent assays - a study based on our endeavors towards assay modernization in the light of quality control tendencies** *Böröcz K, Csizmadia Z, Berki T*
National Community of Young Biotechnologists (FIBOK)	28-29.March, 2018	ELTE, Budapest, Hungary	Poster	**Opening a new door to cost-effective largescale vaccine effectiveness studies** *Böröcz K, Csizmadia Z, Mészáros V, Berki T, Markovics Á, Németh P*
World Immunization Week, International Roundtable Conference	28-30 April, 2018	University of Pécs, Hungary	Presentation	**New challenges: monitoring the long-term efficacy of vaccines** *Böröcz K, Németh P*

MIT-MLDT Congress, 2018 **University of Pécs**	30 August – 1 September, 2018	Pécs, PTE ÁOK	Poster	**Development of a combined high throughput and cost-effective indirect ELISA for MMR vaccine efficacy screening** *Böröcz K, Csizmadia Z, Varga V, Telek V, Berki T, Németh P*
MIT-MLDT Congress, 2018 **University of Pécs**	30 August – 1 September, 2018	Pécs, PTE ÁOK	Poster	**Comparison of chemiluminescence and conventional ELISA techniques in autoantibody detection** *Csizmadia Z, Böröcz K, Telek V, Varga V, Berki T*
5th European Congress of Immunology - ECI Amsterdam	2-5 September, 2018	Amsterdam, Netherlands	Poster	**Development of a robust and standardized immunoserological assay for detection of anti-measles IgG antibodies in human serum** *Böröcz K, Csizmadia Z, Mészáros V, Markovics Á, Farkas K,Telek V, Varga V, Ouma M G, Bodó K, Najbauer J, Berki T B, Németh P*
MIT MLDT **Professional Development Course**	3 October, 2018	Budapest, Hungary	Presentation	**New possibilities of autoantibody detection** *Berki T, Böröcz K*
World Immunization Week, International Roundtable Conference	30 April, 2019	University of Pécs, Hungary	Presentation	**Monitoring of long term efficacy of the MMR vaccination in the Hungarian population** *Böröcz K, Németh P*
1st Pécs-Osijek PhD Symposim	10 May, 2019	University of Pécs, Hungary	Presentation	**Vaccination state of the Hungarian population** *Böröcz K, Németh P*
49th Membrane-Transport Conference	14-17 May, Sümeg	Sümeg, Hungary	Poster and presentation	**A quick trivalent ELISA assay for the detection of MMR vaccine effectiveness, and for seronegativity screening** *Böröcz K, Csizmadia Z,Markovics Á, Farkas K, Najbauer J , Berki T B, Németh P*
WHO Consensus Meeting	8 October, 2019	University of Pécs, Hungary	Presentation	**Conclusions of a measles/MMR serosurvey in the Hungarian population** *Böröcz K, Németh P*
5th International Consensus on ANA pattern (ICAP) Workshop	10-13 September, 2019	Dresden, Germany	Poster	**Comparison of commonly used dsDNA assay types in order to better support an accurate clinical diagnosis** *Böröcz K, Csizmadia Z, Varga V, Berki T* **Evaluation of three years of maternal infertility - related autoantibody test requests** *Csizmadia Z, Böröcz K, Varga V, Balázs N, Berki T*

EWRR	13 February, 2020	Leuven, Belgium	Poster	**Infection (or vaccine)-induced antibody and natural autoantibody levels may show association in systemic lupus erythematosus (SLE) patients** *Böröcz K, Simon D, Erdő-Bonyár S,Csizmadia Z, Kovács A, Czirják L, Németh P, Berki T*
Health Innovation Cluster of Pécs (PEIK) Conference	27 October, 2020	Pécs, Hungary	Presentation	Vaccine Effectiveness Monitoring (early warning) *Böröcz K, Németh P*

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