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Report of the 1st proficiency testing scheme for pulsed field gel electrophoresis (PFGE) typing of Verocytotoxin-producing *E. coli* (VTEC) strains (PT-PFGE1) – 2012-2013

Conducted jointly with the network of public health National Reference Laboratories for VTEC referring to the Food and Waterborne Diseases and Zoonoses Surveillance Programme of the European Center for Disease Prevention and Control

Summary: Although it was considered as non-mandatory, the first EQA scheme on PFGE among the NRLs for E. coli involved 19 NRLs, including 16 EU NRLs representing 13 Member States and the NRLs of Egypt, Norway, and Switzerland. The study was carried out on 11 VTEC strains and the quality of PFGE images was evaluated according to criteria of the PulseNet International Protocol, by assigning ranks from poor to excellent to each of seven parameters. The majority of the NRLs placed between “fair” and “good” in the evaluation of the whole procedure, even though weaknesses in the areas of cells suspension, bands displaying, and DNA degradation were recorded.

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1. INTRODUCTION

Molecular subtyping of bacterial isolates has been successfully applied to the detection of community-wide foodborne disease outbreaks, to aid their epidemiologic investigation, and to facilitate source attribution exercises.

A well-established molecular surveillance network for food-borne infections is *PulseNet International* (www.pulsenetinternational.org), a network of national and regional laboratory networks dedicated to tracking foodborne infections worldwide.

In Europe, the European Centre for Disease Prevention and Control (ECDC) collect molecular typing data, in particular pulsed field gel electrophoresis (PFGE) profiles of Salmonella, *Listeria monocytogenes* and Verocytotoxin-producing *E. coli* (VTEC) strains isolated from human infections.

In 2012, the EC DG SANCO decided to organize the collection of typing data for isolates from food and animals, to improve the surveillance and trace-back of food-borne infections at the national, European and international level, as well as the preparedness to face foodborne outbreaks.

The collection of data has been initially focused on a restricted number of pathogens, namely Salmonella, *L. monocytogenes* and VTEC, and the responsibility of the management of the database on isolates from food and animals was assigned to EFSA, with the scientific and technical support of the relevant EU-RLs.

According to the DG SANCO mandate, the bulk of molecular typing data on food/animal isolates of the selected food-borne pathogens will be primarily produced by the respective networks of National Reference Laboratories (NRLs). Therefore, the EU Reference Laboratory for VTEC (EU-RL VTEC) undertook initiatives to provide specific PFGE training opportunities to the *E. coli* NRLs and to set up an external quality assessment (EQA) program for PFGE.

Such an EQA program initiated in 2013, with the inclusion of PFGE typing in the 10th inter-laboratory study on VTEC identification and typing (PT10), which was performed jointly with the ECDC-FWD network involved in the typing of VTEC strains from human infections. For this purpose, the EU-RL VTEC collaborated with the WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*, Statens Serum Institute, Copenhagen (SSI), which is in charge for the external quality assurance program for the ECDC network of the medical NRLs for VTEC. The aim of such a liaison was the harmonization of the typing methods used by both the NRL networks, to make the

respective monitoring programs and databases compatible for comparison of data referring to human and non-human isolates of VTEC.

As the first EQA scheme on PFGE, the main purpose of the study was the assessment of the level of preparedness of the NRLs network, and the participation of the NRLs in the PT was therefore considered as non-mandatory. The study was carried out on 11 of the 15 test strains sent to the NRLs for PT10. This document represents the evaluation report of the PFGE part of the study.

2. PARTICIPANTS

Nineteen NRLs, including 16 EU NRLs representing 13 Member States and the NRLs of Egypt, Norway, and Switzerland, participated in the study.

Each NRL received its own individual laboratory numerical code, which is reported in the result tables.

The NRLs participating in the study were:

- Belgium, Scientific Institute of Public Health
- Czech Republic, Veterinary Research Institute
- Denmark, National Food Institute, Technical University of Denmark
- Egypt, Central Public Health Laboratories of Ministry of Health and Population, Cairo
- Finland, Finnish Food Safety Authority Evira, Helsinki
- Finland, Finnish Food Safety Authority Evira Kuopio
- France, VetAgro Sup Campus Vétérinaire de Lyon
- Germany, Federal Institute for Risk Assessment (BfR)
- Greece, National School of Public Health, Central Laboratory of Public Health
- Ireland, Central Veterinary Research Laboratory
- Italy, Istituto Superiore di Sanità
- Lithuania, National Food and Veterinary Risk Assessment Institute
- Norway, Norwegian Veterinary Institute
- Poland, National Veterinary Research Institute, Pulawy
- Slovakia, Public Health Authority, UVSZR
- Slovakia, State Veterinary and Food Institute Dolný Kubín
- Spain, Laboratorio Central de Sanidad Animal
- Spain, University of Santiago de Campostela, Dept. of Microbiology and Parasitology, Lugo
- Switzerland, University of Zurich

3. MATERIALS AND METHODS

The test material sent to the NRLs was constituted by 11 strains of *E. coli* (samples 1 to 11), provided as soft agar stab cultures.

3.1. Sample preparation

The test *E. coli* strains were selected among those present in the SSI reference collection. They were prepared at SSI in the period between 12 and 21 November, with the assistance of a scientist from the EU-RL VTEC. They consisted of bacterial cultures seeded by stabbing into soft (0.3 %) nutrient agar in plastic vials. The cultures were incubated 18 hours at 37 °C ± 1 °C and labelled with randomly generated numerical codes (3 or 4 digits), different for each NRL. The test samples were shipped between 27 November and 4 December by courier.

3.2. Collection and evaluation of the NRL results

The NRLs were requested to use the laboratory procedure in use in the PulseNet international network (available at URL:
http://www.pulsenetinternational.org/assets/PulseNet/uploads/pfge/PNL05_Ec-Sal-ShigPFGEprotocol.pdf) and to submit by email the pictures of the PFGE gels as TIFF files together with a scheme of sample loading in a separate word/excel file.

The quality of PFGE images was evaluated according to criteria defined in the “Standard Operating Procedure for TIFF Quality Grading” of the PulseNet International Protocol (PNQ01). The same criteria are adopted by the ECDC for evaluating the quality of gels of human strains submitted to the TESSy molecular surveillance system. The evaluation criteria are reported in Table1.

The performance of each NRL was evaluated by considering the quality grading in use at PulseNet, which assigns ranks from poor to excellent to each parameter (Table 1). Each grade has been transformed into a score from 1 to 4 according to the following scheme:

1: Poor

2: Fair

3: Good

4: Excellent

The average score for each parameter was calculated based on the grading obtained by all the 19 participating laboratories and a threshold of three points was set for a satisfactory performance in each parameter. Given the complexity of the inter-laboratory trial and the non-mandatory nature of this first PT on PFGE typing, an overall threshold for the identification of the laboratories underperforming in the whole procedure was not

established. Rather, the poor-to-fair performance of the laboratories was assessed for each of the different sections of the typing method, according to the parameters under evaluation. The purpose of such an evaluation was assessing the level of preparedness of the network and refining the specific training program of the EU-RL VTEC for 2014.

The sum of the points obtained for all the parameters originated a general score anyway, but it was only included in the individual reports sent out to the laboratories for self-evaluation purposes, together with an overall comment containing suggestions on how to improve the quality of the images, with respect to the specific points that generated poor performance.

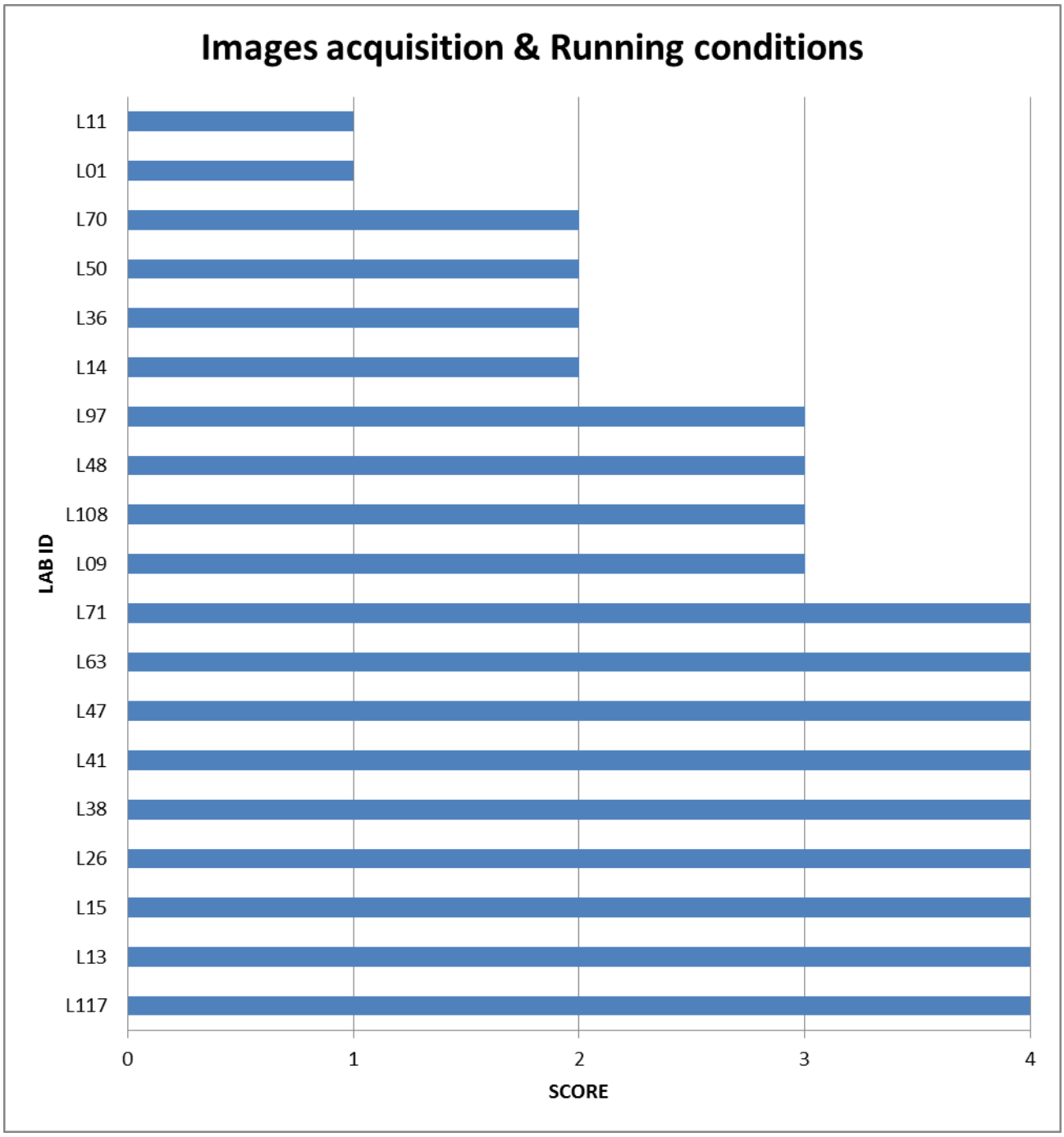
Table: Quality grading of PFGE images according to the PulseNet guidelines

Parameter	Image Quality Grading Guidelines			
	Excellent	**Good**	**Fair**	**Poor**
Image acquisition and running conditions	As per PulseNet protocol: Gel fills whole TIFF. Wells included on TIFF. Bottom band of standard is between 1.0 cm and 1.5 cm from the bottom of the gel.	Gel does not fill whole TIFF but band finding is not affected.	Not protocol: only one of the following: Gel does not fill whole TIFF, and band finding is affected. Wells not included on the TIFF. The bottom band of a standard is not between 1.0 and 1.5 cm from the bottom of the gel. Band spacing of standards does not match the global standard.	Not protocol: more than one of the following: Gel doesn't fill whole TIFF and this affects band finding. Wells not included on TIFF. The bottom band of a standard is not between 1.0 and 1.5 cm from the bottom of the gel. Band spacing of standards does not match the global standard.
Cell suspensions	The cell concentration is approximately the same in each lane.	Up to two lanes contain darker or lighter bands than the other lanes.	More than two lanes contain darker or lighter bands than the other lanes, or at least one lane is much darker or lighter than the other lanes, making the gel difficult to analyse.	The cell concentrations are uneven from lane to lane, making the gel impossible to analyse.

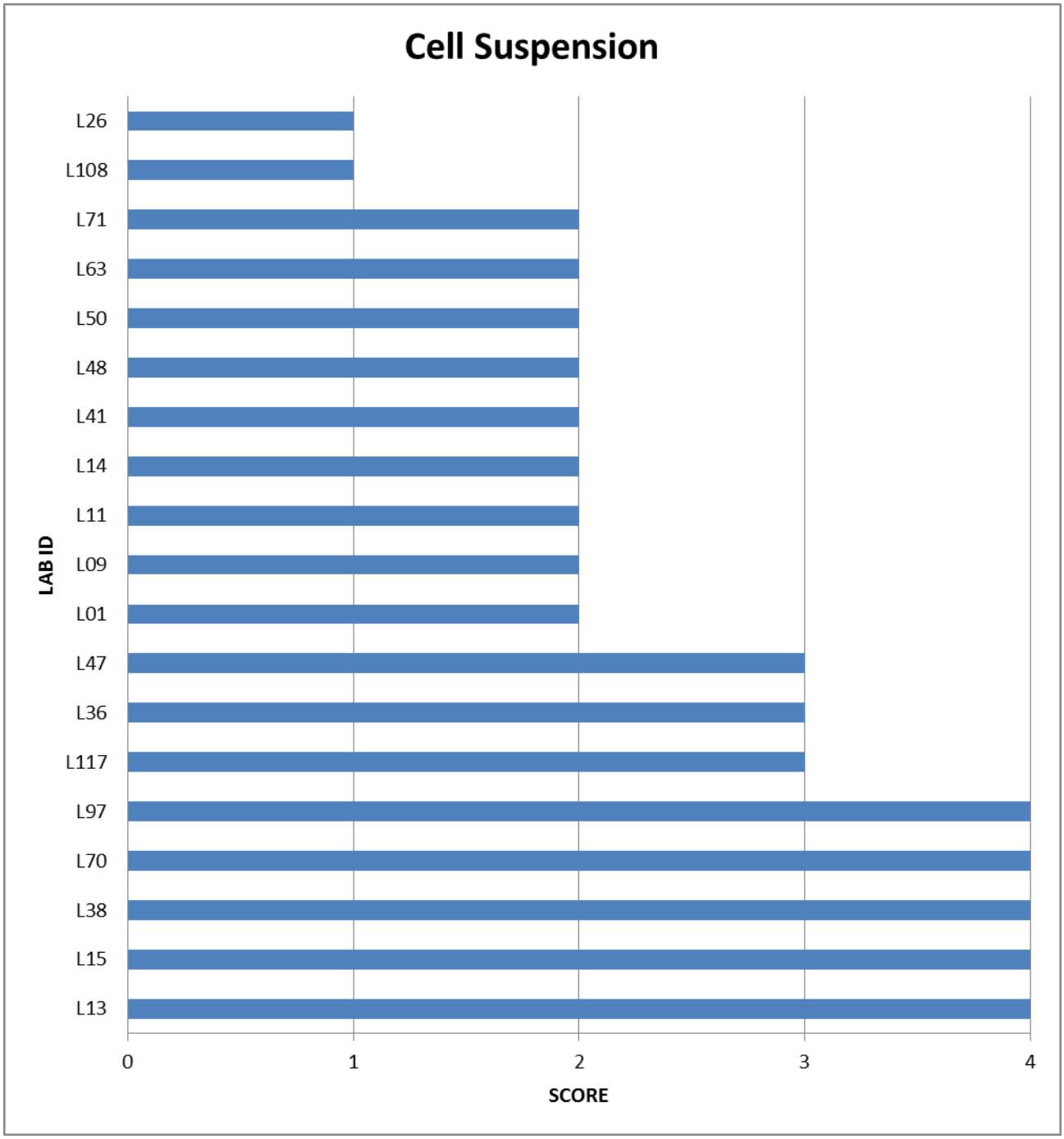
Parameter	Image Quality Grading Guidelines			
	Excellent	**Good**	**Fair**	**Poor**
Bands	Clear and distinct all the way to the bottom of the gel.	Slight band distortion in one lane, but this does not interfere with analysis. Bands are slightly fuzzy and/or slanted. A few bands (three or less) are difficult to see clearly (DNA overload), especially at bottom of gel.	Some band distortion (e.g., nicks) in two to three lanes but still analysable. Fuzzy bands. Some bands (four to five) are too thick. Bands at the bottom of the gel are light, but analysable.	Band distortion that makes analysis difficult. Very fuzzy bands. Many bands too thick to distinguish. Bands at the bottom of the gel too light to distinguish.
Lanes	Straight.	Slight smiling (higher bands in the outside lanes than on the inside). Lanes gradually run longer toward the right or left. Still analysable.	Significant smiling. Slight curves on the outside lanes. Still analysable.	Smiling or curving that interferes with analysis.
Restriction	Complete restriction in all lane.	One to two faint shadow bands on the gel.	One lane with many shadow bands. A few shadow bands spread out over several lanes.	Two or more lanes with several shadow bands. Lots of shadow bands over the whole gel.
Gel background	Clear.	Mostly clear background. Minor debris present that does not affect analysis.	Some debris present that may or may not make analysis difficult (auto band search finds too many bands). Background caused by photographing a gel with very light bands (image contrast was “brought up” in photographing gel-makes image look grainy).	Lots of debris present that may or may not make analysis difficult (auto-band search finds too many bands).
DNA degradation (smearing in the lanes)	Not present.	Minor background (smearing) in a few lanes but bands are clear.	Significant smearing in one or two lanes that may or may not make analysis difficult. Minor background (smearing) in many lanes.	Significant smearing in more than two lanes that may or may not make analysis difficult. Smearing so that a lane is not analysable (except if untypeable [thiourea required]).

4. RESULTS

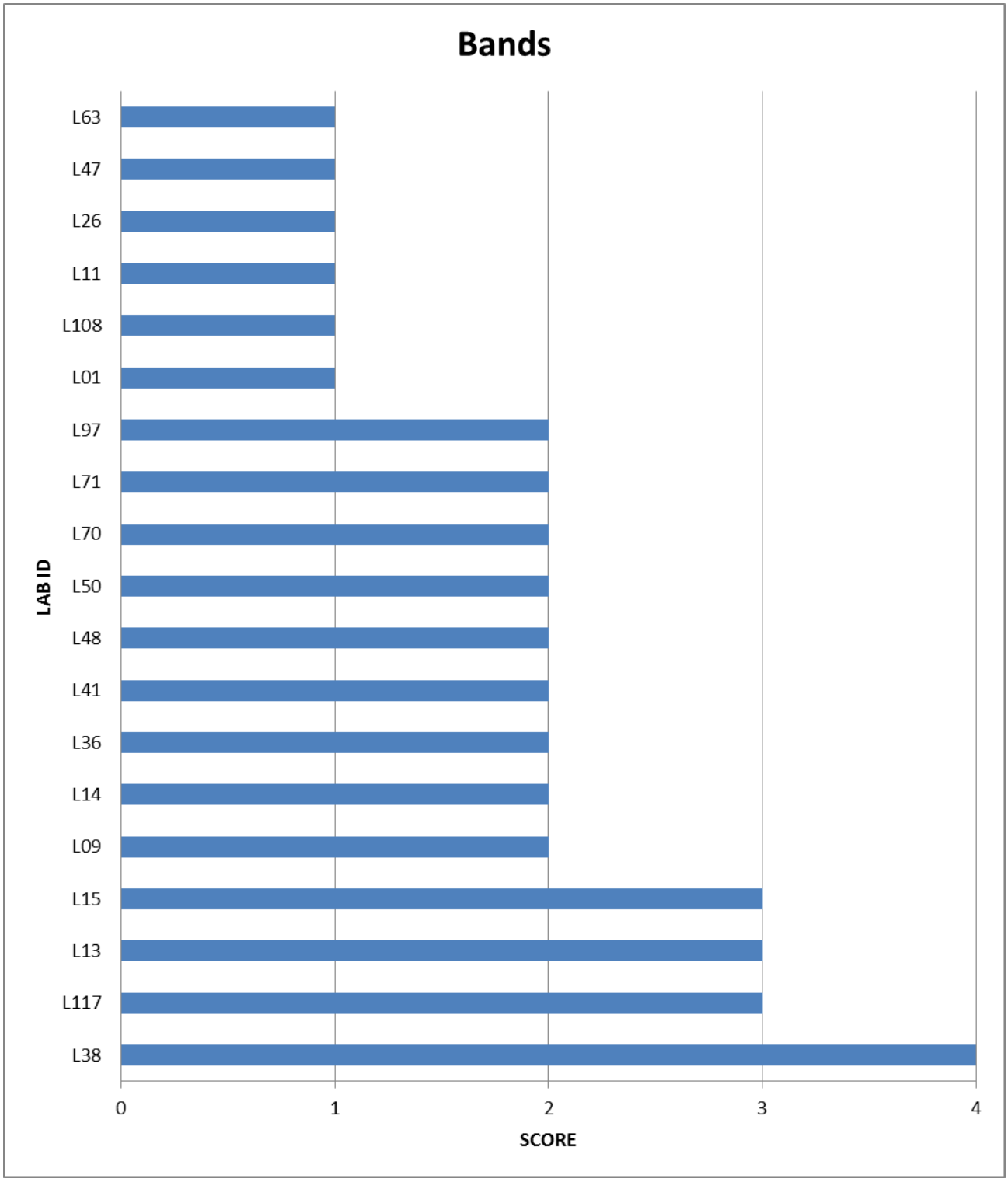
The study was carried out by 19 NRLs. The score obtained by each laboratory is reported in the figures below, broken down by parameter.



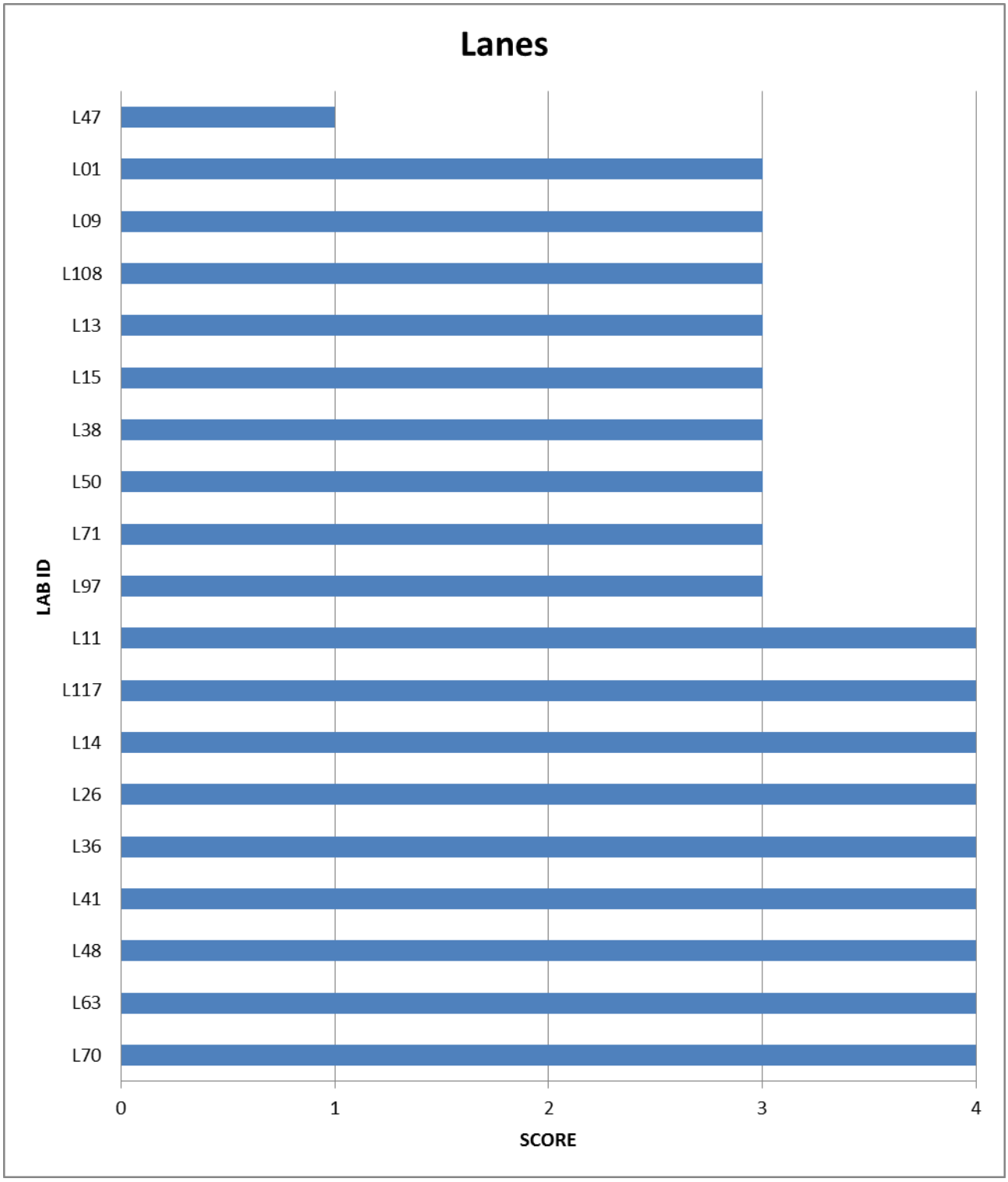
The mean score obtained by the participant laboratories for the parameter “image acquisition and running conditions” was 3.05.



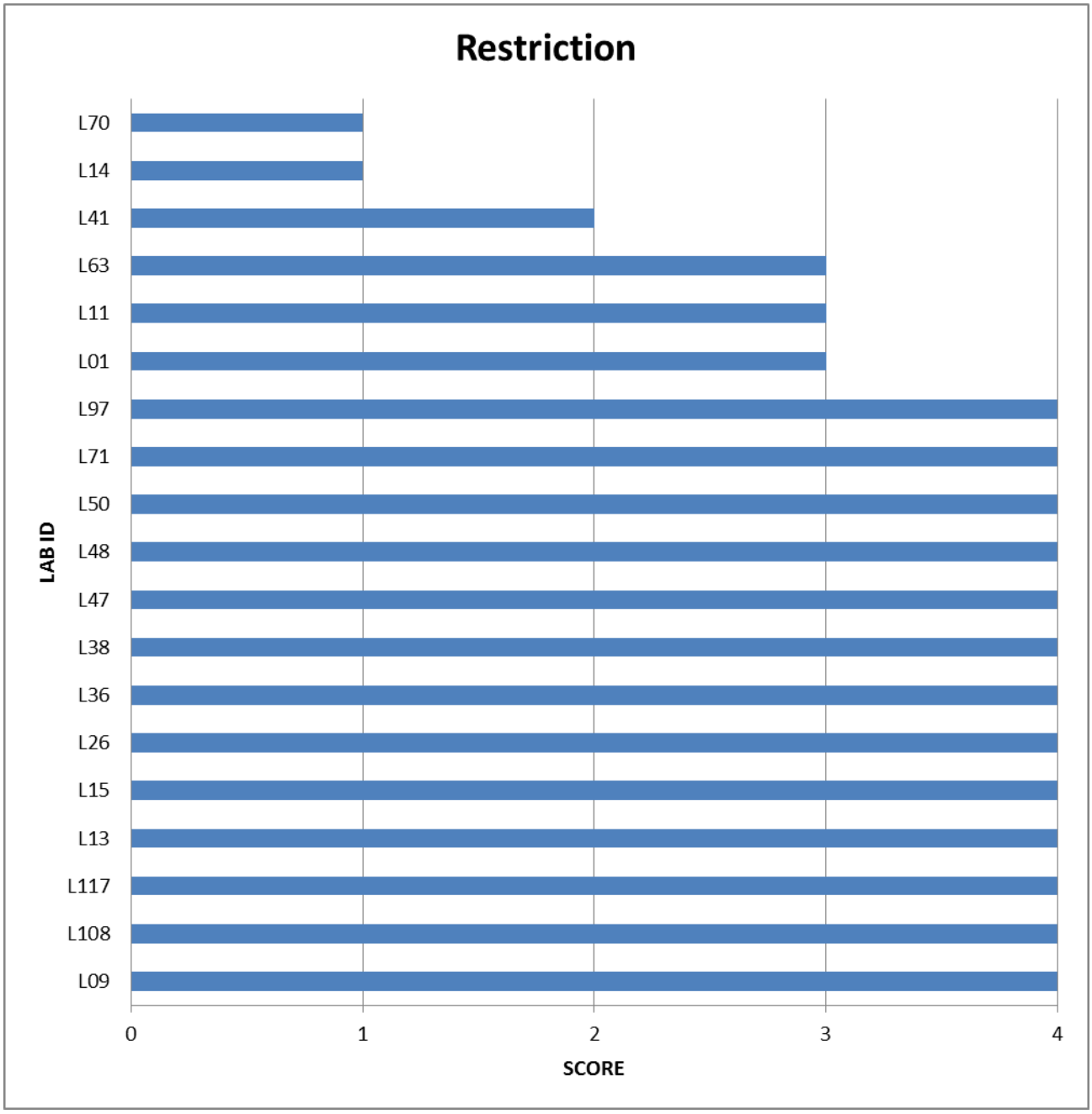
The mean score obtained by the participant laboratories for the parameter “cell suspension” was 2.57.



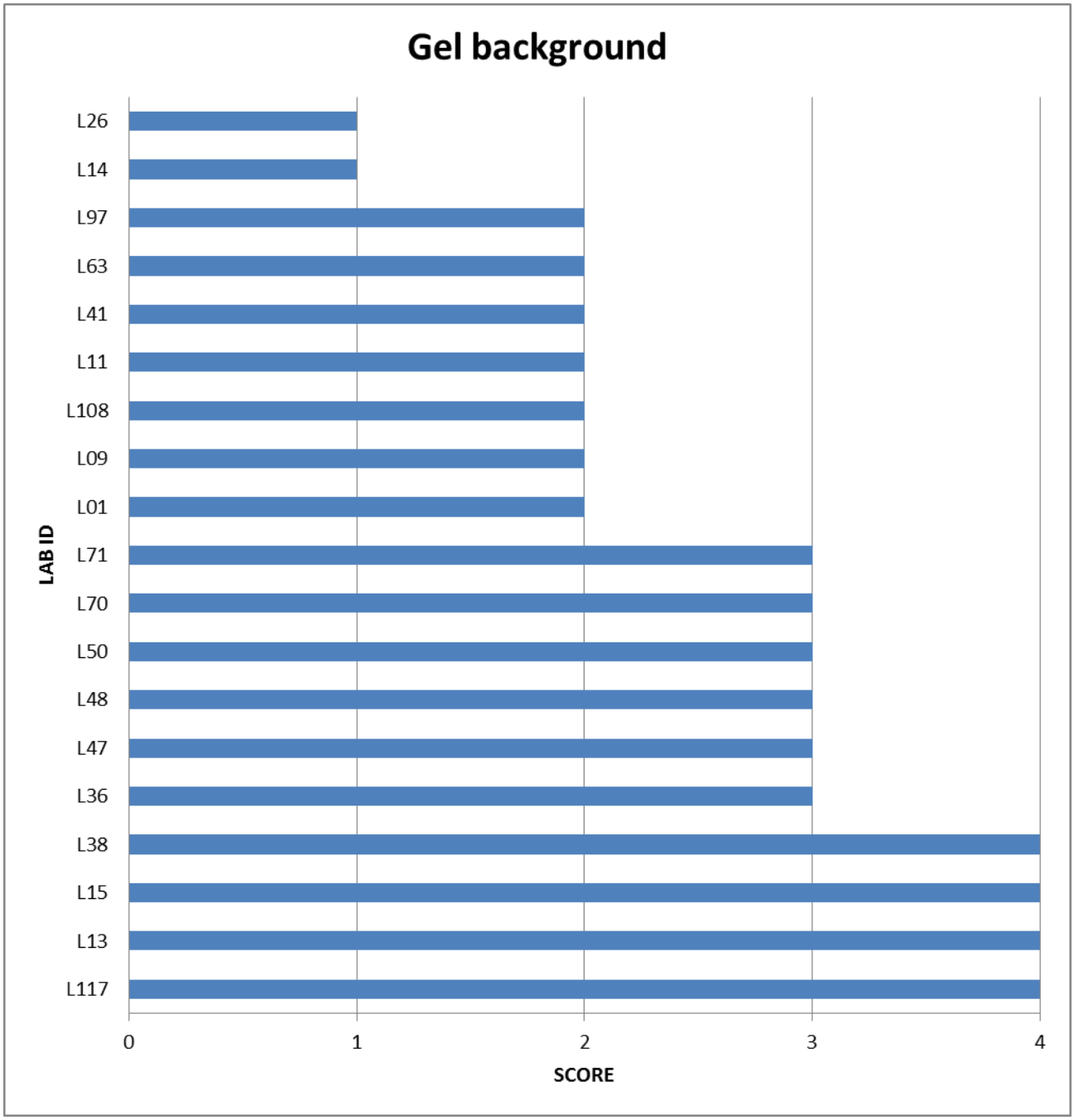
The mean score obtained by the participant laboratories for the parameter “bands” was 1.94.



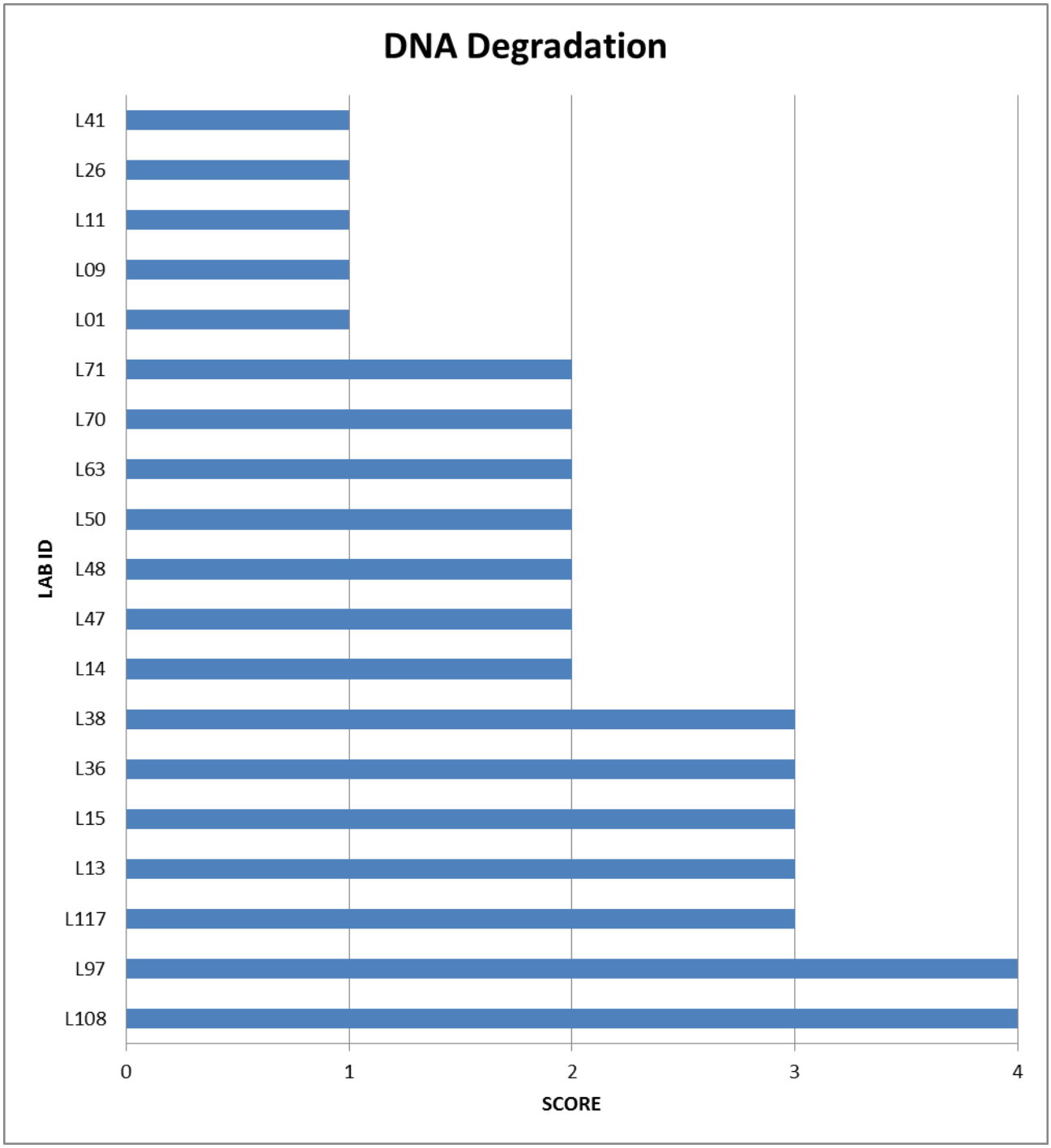
The mean score obtained by the participant laboratories for the parameter “lanes” was 3.36.



The mean score obtained by the participant laboratories for the parameter “restriction” was 3.42.



The mean score obtained by the participant laboratories for the parameter “gel background was 2.63.



The mean score obtained by the participant laboratories for the parameter "DNA degradation was 2.21.

7. CONCLUDING REMARKS

PFGE typing of VTEC and other foodborne pathogens is a complex laboratory procedure, potentially affected by several troubles. The protocol used in this 1st EQA for PFGE typing of VTEC strains from non-human sources is the same used for producing PFGE profiles of human strains in the ECDC-FWD network of public health NRLs and in the international

worldwide network PulseNet. Therefore, its adoption represents the key to the harmonization of the forthcoming molecular database for strains from food and animals, which will be managed by the European Food Safety Authority (EFSA) under the auspices and the mandate of the EC DG SANCO, with the other above mentioned networks.

For the evaluation of such a complex procedure, seven parameters have been identified, representing good indicators of the critical points for producing PFGE profiles suitable for comparison and searches against a database of profiles produced by different operators. In this 1st EQA on PFGE typing, the analysis of the PFGE profiles provided by the participant NRLs was conducted according to such parameters and, in general, it highlighted a good technical level of the participating laboratories. However, the existence of areas for improvement was identified for some of the sections of the protocol used for plug preparation and sample run.

Generally, the laboratories that voluntarily participated in this 1st EQA on PFGE typing of VTEC from non-human sources responded quite satisfactorily, with the majority of them placed between “fair” and “good” in the evaluation of the whole procedure. Nevertheless, the average score assignment for each parameter showed that many of the participating laboratories displayed weaknesses in the areas of cells suspension, bands displaying, and DNA degradation, indicating these points as the most compelling to be treated in the next training sessions.

All the participant laboratories received individual reports, containing a detailed evaluation of the critical point in their own gels and a general comment with suggestions for improving the performance.

In conclusion, this 1st EQA on PFGE typing of VTEC from non human sources provided useful information on the capability of the network to produce PFGE profiles suitable for the inclusion in the forthcoming molecular database managed by EFSA, for the purpose of conducting cluster analyses and comparison with analogue profiles of VTEC isolated from human sources and collected within the ECDC-FWD network. Moreover, the deep analysis of the results obtained in this PT will allow a refinement of the EU-RL VTEC training programs, in order to place the network of *E. coli* NRLs in the position of contributing PFGE profiles of VTEC strains from food and animals suitable for the intended purposes of detecting community-wide foodborne disease outbreaks, facilitating source attribution exercises, and to contribute in general to epidemiologic analyses.

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